



XX INTERNATIONAL CONGRESS OF ENTOMOLOGY



Firenze, Italy, August 25 - 31, 1996

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EDITORIAL NOTES

This book contains the complete texts of the Plenary Lectures and the abstracts of the oral and poster communications presented at the XX International Congress of Entomology.

All the abstracts have not been edited by the Scientific Secretariat and are printed as received.

The abstracts are grouped by sections and are in progressive order. The number of the abstract is composed of two parts: the first two figures indicate the section, the last three figures are the order number. The same reference number is indicated in the programme of the congress.

The reference number of the poster presentations will also appear on the poster board.

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AN OUTLINE OF THE HISTORY OF ITALIAN ENTOMOLOGY

Baccio Baccetti

Istituto di Biologia Generale e Centro CNR
per lo Studio delle Cellule Germinali, Siena, Italy

"Italy was early the home of many famous naturalists and physicians" Howard (1930) wrote in his "A history of applied entomology" "and in entomology has produced many well known workers". At that time such an assertion was easy and practically due.

Entomology, originated in the hands of the Ellenic scientists like Aristoteles (384-322 b.C.), Theophrastus (371-286 b.C.), Dioscorides (1 century a.C.) and the Carthaginiensis Magon, brother of Hannibal (II century a.C.), consisted in a mere reperitory of the generic wasps, ants, locusts, cycads, beetles, flies, mosquitos, louses and cymexes, devoid of any morphological distinction, but it was essentially on the honey bee that some superficial attention was spent. The bee was described without any real observation on the mysterious biology of the insect, except the aristotelic distinction in three castes, tough uncorrectly interpreted. Magon, indeed, added the peculiar theory of the origination of bees from decomposed cadavers.

In the same centuries, in Italy, the Roman Marcus Porcius Cato (234-149 b.C.) wrote "De agricultura", wich included some entomological observations, and the Reatine Marcus Terentius Varro (116-27 b.C.) in his book "Rerum rusticarum", (37 b.C.), was the first latin georgic author to discuss about the honey bees, adding practical suggestions to the aristotelic treatise, mainly obtained from the Italian apicultural experience. Varro therefore prepared the material for the fourth "Georgica" (37-30 b.C.) of Publius Vergilius Maro (70-19 b.C.), conserving the method of Magon for the production of bees from putrified cadavers, and extending studies on the many pests of the bees. The subject was later treated also by Junius Moderatus Columella (I century a.C.), who devoted to apiculture most of the ninth volume of "De re rustica libri XII". In the same century, Caius Plinius Secundus (Plinius Major, 23-24 a.C.-79 a.C.) wrote "Naturalis historia libri XXXVII". With this book the apiculture was reduced to a part of the chapter on small animals ("animales parvi"), which, with "terrestres", "aquatici" and "volucres", formed the four animal classes of his classification. Following Aristotle, Pliny listed in the class "parvi" also few coleopterans, orthopterans, hymenopterans, dipterans, hemipterans without further distinction. The same information were reported by Claudius Aelianus (170-249 a.C.) in his "De vi et natura animalium", while the georgic Rutilius Taurus Palladius (IV century a.C.) synthesized the apiculture of Columella in his "Opus agriculturae".

For nearly the entire millennium to follow nothing new happened in Italian entomology, while the aristotelic Arabs Mesue (?-857), Avicenna (980-1037), Averroes (1126-1194), and the scholastic Europeans Thomas of Cantimpré (1186-1263), Vincent of Beauvais (?-1264), and Albertus Magnus (1193-1280) translated and commented Aristotle. The only Italians were Piero de' Crescenzi (Bologna, 1230-1321) who in his treatise "Il libro dell'agricoltura", published in Florence, 1478, reported the usual aristotelic apiculture and Michelangelo Tanaglia (Florence,

1437-1512) with "De Agricultura" vol. III "Delle Ape" (manuscript). But in the same period entomology extended to the silk moth, the Bombyx. This novelty happened in an anonymus small book, written in Italian around 1473, and presently conserved in the form of manuscript in the National Library of Florence. I have found and published this paper in 1965, under the title "Trattatello di apicoltura, del porre i mori e del porre i bigatti". It is the first book of entomology ever to be written.

In the subsequent century the situation was more or less the same. The Italian Gerolamo Cardano (1501), from Pavia, author of "De subtilitate libri XXI" (1558), Giulio Cesare Scaligero, from Verona (1484-1558), who commented Aristotle (1519), and Piero Andrea Mattioli, from Siena (1500-1577), who commented Dioscorides (1568), mentioned only, supporting their arguments with information taken from the tradition, honey bees, locusts, cicads and few other insects. In addition we must remember the Florentine Giovanni Rucellai (1475-1525) for a short poem in Italian, "Le api" (1568) and two other compositions, both in Latin, both on the silk moth: "Opusculum de Bombyce" (1493) of Ludovico Lazzarelli (1450-1500), and "De Bombycum cura et usum" (1527) of Marco Girolamo Vida (1490-1566). The value of these compositions is rather poor. All the authors were under the complete influence of Vergilius and of Pliny: Vida, e.g., suggested a personal modification to the technology for the production of bees from a putrified cadaver, adapted to the silk moth.

At the beginning of the seventeenth century, the first true Italian entomologist to publish a book on the topic was Ulisse Aldrovandi (1522-1605) from Bologna, doctor in medicine, professor of Bologna University, author of "De animalibus insectis libri septem" (Bologna, 1602) who reports all the aristotelic, plinian and vergilian data, without criticizing them, but adding personal observations gathered in half a century of travels, collections, experimental breeding. At the same time a Neapolitan, Ferrante Imperato (1550-1635), published an enormous volume "Dell'istoria naturale libri XXVIII" where he reported and commented the specimens, including insects, of his museum.

The century was preparing, indeed, the great scientific Galileo's revolution. While the Florentine T. Albmain, in his treatise on the "four elements", explained in 25 lessons, repeated, still in 1668, the aristotelic information, four major scientists abandoned the repetitive classic procedure of the previous centuries and used the galileian experimental method and the galileian instrumentation, under the influence of the "Accademia dei Lincei", that in those very years was to be founded (1603), in Rome. The Tuscan Galileo (1564-1642), elected in 1611, was in fact one of the first fellows of the Academy; he sent a microscope to the academic princeps Federico Cesi (1585-1630) as a gift. This instrument, named by Galileo "occhialino" had been realized by himself and experimented in the examination of a flea, which resulted (as defined in a letter to Cesi, still conserved in the Academy) "orribilissima". In fact, Cesi already knew a

"tubum opticum" given by "duobus germaniis.... artificibus fabrisque" to the Lyncaeus Joannes Faber, an instrument that Faber himself named "microscopium" and experimented on a louse. Galileo, during his stay in Padua, heard about the engine and assembled one "sua sponte, non visu prius tubo similem". The two Germans, in turn, had been stimulated by Joannes Keplerus, who had read the theoretical bases in the volume "De refractione" of Giovan Battista Della Porta (1530?-1615), Neapolitan Lyncaeus. In conclusion: Della Porta had the idea, Keplerus transmitted it to the Germans, the Germans realized the instrument, Faber got it and wrote about the engine, simultaneously Galileo heard the story and contemporarily assembled a similar instrument and sent it to Cesi. The circle of this invention starts and stops in the range of "Accademia dei Lincei".

The four revolutionary entomologists were Francesco Stelluti, Francesco Redi, Pietro Paolo Da Sangallo and Marcello Malpighi. Francesco Stelluti from Fabriano (1577-1653), one of the founders of the "Accademia dei Lincei", had the privilege of working with Galileo's microscope and became the first microscopist in entomology, detecting the minute morphology of the honey bee (published in the ccsian "Apiarium" in 1625) and of Calandra granaria (published in the "Perseo" in 1630). Francesco Redi from Florence (1626-1698), physician at the court of Medici and one of the founders of the Florentine "Accademia del Cimento", experimented on the insect reproduction (1668, 1684) and was the first to exclude the spontaneous generation. This conclusion was substantiated by his Florentine pupil P.P. Da Sangallo (?-1670), who detected the reproduction and metamorphosis of the mosquitoes of Florence (1679). Both Redi and Da Sangallo used the microscope, as a multitude of investigators did in the whole Europe in the second half of the century. But the fourth great entomologist and major microscopist of the seventeenth century was Marcello Malpighi from Bologna (1628-1694), one of the founders of "Accademia del Cimento", who published the famous "Dissertatio epistolica de Bombyce" (London, 1669), where he described the excretory tubules, and in which is included the chapter "De Gallis" in "Anatome Plantarum" (1679), where he found the cecydology. Two other microscopists of this century, even if they were not real entomologists, deserve to be mentioned, and both being important ecclesiastic authorities: the Sicilian G.B. Hodierna (1597-1660), who published a small paper on the anatomy of the eye in the insects ("L'occhio della mosca", Palermo, 1644) and the late aristotelic, but precise morphologist Filippo Buonanni (Rome, 1638-1725) an outspoken enemy of Francesco Redi, who was one of the last epigones of the spontaneous generation. Buonanni in "Observationes circa viventia, quae in rebus non viventibus reperiuntur" (1691), revealed the morphology of many insect mouth apparatuses and described the first Thysanopteron.

Of minor importance, the applied entomologist Paolo Boccone (Frate Silvio), from Palermo (1633-1704).

The eighteenth century opens with a great entomologist, Antonio Vallisnieri (Lucca 1661-1730), professor in Padua, who in an enormous series of treatises and articles (all published from 1700 to 1733), reports important ethological observations on a number of injurious or parasitic insects and proposes a new kind of classification for the class. But in this century, when the great European taxonomists edify the system of insects, and the new continents are explored by travelling entomologists, the Italian supremacy in this science starts to decline. In the early years of this century we have only two marine biologists, Diacinto Cestoni (Livorno, 1637-1718), and Luigi Ferdinando Marsili (Bologna, 1658-1730), who wrote observations on the Kermes coccids. In the rest of the century a number of insect collectors

wrote faunistic descriptions of their countries: Michele Giorna (?-1799), Carlo Allioni (1728-1804) and Leonardo De Prunner in Piedmont, the Trentine G.A. Scopoli (1723-1788) in Carniola (1763) and in Insubria (1786-1788), the Florentine applied naturalist Giuseppc Del Papa (1648-1735) in coastal Tuscany, the Ravennate orthopterologist Giuseppe Zinanni (1692-1753) in Romagna, the Florentine Pietro Rossi (1738-1804), professor of "Insettologia", in the University of Pisa and, therefore, the first chairman of entomology in the world, in "agro florentino et pisano" (1790, 1792-94), the Neapolitan Vincenzo Petagna (1734-1810), in Calabria (1787), Domenico Cirillo (1734-1799) in Naples, and Filippo Cavolini (1756-1810), who studied the insects of the fig, also in Naples. But outstanding biologists of the caliber of the various Redi or Malpighi are no more present in Italian entomology.

In the nineteenth century two new phenomena became evident: the formation of the professional schools, usually around Universities or Museums, and, in the second half of the century, the constitution of the national societies. As far as entomology is concerned, we have already seen that in the eighteenth century, a similar tradition had started in Turin, Florence, Naples. These three important cities continued to produce a number of professional entomologists during the subsequent two centuries.

Turin, in the Institute and Museum of Zoology of the University, generated a famous entomological school, starting from the coleopterist Franco Andrea Bonelli (1784-1830), a former student of Lamarck, and continuing with the applied entomologist and traveller Giuseppe Gené (1800-1847), the museologist traveller Vittore Ghiliani (1812-1878), and the embryologist Filippo De Filippis (1814-1877) (one of the first Italian darwinists), the three famous dipterologists Luigi Bellardi (1818-1889), Ermanno Giglio-Tos (1865-1926) and Mario Bezzi (1868-1927), with the coleopterist Eugenio Sella (1820-1882), the hemipterist amateur Antonio Garbiglietti (1807-1887), the insect anatomist Lorenzo Camerano (1856-1919), the traveller and collector Enrico Festa (1868-1939), the orthopterists Achille Griffini (1870-1932) together with the already mentioned Giglio-Tos, the hymenopterist Giovanni Gribodo (1846-1924), the dermapterist Alfredo Borelli (1858-1943), the lepidopterist Ubaldo Rocci (1885-1943) and the pathologist and parasitologist Edoardo Perroncito (1847-1936). In the present century most of the entomological activity in Turin was carried out by Athos Goidanich (1905-1987), the founder of the Institute of Agricultural Entomology of that University, an eclectic taxonomist and ethologist of many important injurious orders, and by his school. Also the coleopterist Giuseppe Della Beffa (1885-1969) did a rich applied research in the Phytopathology Observatory and wrote a very successful treatise on injurious insects.

In Florence most of the entomological activity was practiced in the famous "Imperial Regio Museo" called "La Specola". The first entomologist there had been the applied coleopterist Carlo Passerini (1793-1857), emerging out of a small group of minor investigators. Finally the Florentine naturalist Bettino Ricasoli (1809-1880) became President of the Government, at the time of Florence capital of Italy, and created the first chair of Zoology in the new born "Istituto di Studi Superiori", covered by the first prestigious Florentine modern entomologist Adolfo Targioni Tozzetti (1823-1902). Targioni was a great scientist and manager. Taxonomist (coccids, aphids, orthopterans), museologist, traveller and applied entomologist. Founder and first president of the "Società Entomologica Italiana" (Florence, 1869), founder and first director of the governmental "Stazione di Entomologia Agraria" (Florence, 1875), the first institution of this kind in Europe. Targioni had a large school of students and associates:

the lepidopterist Pietro Stefanelli (1835-1919), the coleopterists Vittorio Pecchioli (1790-1870), Pietro Bargagli (1844-1918), and Angelo Senna (1866-1952), the collectors Guelfo Cavanna (1850-1920) and Ferdinando Piccioli (1821-1900), the muscologist coleopterist Antonio Carruccio (1839-1923), the aphidologist Gustavo Del Guercio (1863-1954), and, above all, the encyclopedic genius Antonio Berlese (1863-1927), who was to be his successor in directing the "Stazione". In this group must be mentioned also the orthopterist and muscologist Apelle Dei from Siena (1819-1903), the zoologist Eugenio Ficalbi (1858-1922), professor in Pisa, where he published excellent papers on mosquitoes, and the insect anatomist Pio Mingazzini (1864-1905), professor of Zoology in Florence. Berlese, born in Padua, was an excellent anatomist and a histologist of insects, a modern applied entomologist, who discovered new formulations of insecticides and efficiently practiced the biological control, a major acarologist and author of a monumental treatise of entomology. Founder of the entomology journal "Redia" (1903) he had a large school of students and associates, including some amateurs: the great entomologist Filippo Silvestri (1873-1949), his former assistant in the Entomology Laboratory of Portici, the amateur coleopterist Giuseppe Leoni (1866-1928), the forest entomologist Giacomo Cecconi (1866-1941), the orthopterist Guido Paoli (1881-1947), the lepidopterist Roger Verity (1883-1959), the applied entomologists Ettore Malenotti (1887-1949), Costantino Ribaga (1870-1945), Leopoldo Chinaglia (1890-1916) and Antonio Melis (1891-1963), who was also an appreciated thysanopterist and became the successor of Berlese in the direction of the "Stazione di Entomologia Agraria". He also founded in 1950 the "Accademia Nazionale Italiana di Entomologia", together with Goidanich, Grandi and Grandori. Successor to Melis in the Direction of "Stazione" was Rodolfo Zocchi (1922-), forest entomologist.

The school of Naples was not of minor importance. In nineteenth century entomology flourished under the dynasty Costa: Oronzio Gabriele Costa (1787-1867) and his son Achille Costa (1823-1898) wrote on various entomological topics and published other studies on insects injurious to the olive tree. The Costas wrote together a splendid "Fauna del Regno di Napoli" and Achille, alone, an enormous "Geofauna Sarda". Another son of Oronzio, Giuseppe Costa, worked in Lecce on the fauna of Terra d'Otranto (1871). In the years 1890-1903, the already mentioned Antonio Berlese, inaugurated the chair of Entomology of the University of Portici and had, as his first important pupil, Filippo Silvestri who succeeded to him when the master left Naples for Florence. Silvestri was an outstanding scientist. A taxonomist and morphologist in a large number of orders, discovered and described an enormous amount of insects and myriapod species, genera, families, orders, all new for science. He was one of the major experts in the biological control by parasitoids in the world, he created the modern style for the monographical study of the single injurious species (taxonomy, morphology of all stages, ethology, control, list of parasitoids with their morphology and reproductive biology). He was also a great traveller and initiated an important journal, at present named "Bollettino del Laboratorio di Entomologia Agraria" of Portici, and a splendid treatise of applied entomology. He was also a major Master: his school still includes many of the most active Italian agricultural entomologists. Among his former students and associates we must remember the coccidologists Gustavo Leonardi (1869-1918) and Vincenzo Lupo (1908-), who started a nice school in Catania, the chalcidologist Luigi Masi (1879-1961), the applied entomologists Giovanni Martelli (1877-1954) and Giuseppe Russo (1897-1971), the orthopterist Giuseppe

Jannone (1907-1971), the aphidologist Domenico Roberti (1912-), who had a valid school in Bari, and many others. But also the Institute and Museum of Zoology of the University of Naples continued to have good entomologists. Ernesto Caroli (1878-1958) studied Collembola, Umberto Pierantoni (1876-1959) was a learned specialist of the endosymbiosis in insects and Director of the Institute; after him the orthopterists Mario Salfi (1900-1970), who succeeded him on the chair and Marcello La Greca who started a sound school in the Institute of Zoology of Catania. The last one was a well recognized expert in insect biogeography and morphology, and gave the first description of the resilin. Another member of the same Neapolitan school has been the endocrinologist Baldassarre De Lerma (1908-1996) who discovered the corpora cardiaca. But in addition to Turin, Florence and Naples, which for several reasons had maintained for a long time the leadership in entomology, other schools became very active in the last two centuries.

In the nineteenth century the pathologist of the silk worm Carlo Agostino Bassi (1773-1856) worked in Milan, while the amateur coleopterist Antonio Villa (1806-1895) and his brother Giovanni associated with the group of Florence in the foundation of the "Società Entomologica Italiana". Felice Franceschini (1854-1928), school professor of applied entomology in Milan, published in 1891 a book on "Insetti Nocivi" and many papers of the silk moth. Eugenio Bettoni (1845-1898) wrote a book on the fauna of Brescia. Paolo Magretti (1854-1913) was a good traveller hymenopterist amateur, and Emilio Turati (1858-1938) a famous lepidopterist. More recently the coleopterist and biogeographer Mario Magistretti (1902-1974) was one of the more relevant investigators in modern taxonomy.

In the same time an important school, essentially specialized in the study of the silk moth, arose: the already mentioned C.A. Bassi, Emilio Cornalia (1824-1882) and Remo Grandori (1885-1955), a pupil of Battista Grassi (see below), were authors of important handbooks on the Bombyx. Grandori, with his wife Luigia, created an important school, whose major exponent is now the chalcidologist Giorgio Domenichini, professor in Piacenza. Connected to the Museum of Natural History of Milan Lucio Micheli (1887-1951) studied the biology of Apidae. Later Cesare Conci (1920-) covered the position of Director of the Museum and that of President of the "Società Entomologica Italiana" being an expert in Anoplura, Odonata and Psylloidea.

In Bergamo Antonio Curò (1828-1906), lepidopterist, published an appreciated catalogue on Italian Lepidoptera.

In Pavia, where Scopoli had been very active in the eighteenth century, the professor of the Agriculture in the University Giuseppe Bayle-Barelle (1768-1811) published an useful book on injurious insects (1809) and also the professor of Zoology Giuseppe Balsamo-Crivelli (1800-1874) worked in applied entomology while the school professor Angelo De Carlini (?-1811) wrote on Orthoptera and Hemiptera. More recently a member of the Roman school of Battista Grassi (see below), Carlo Jucci (1897-1962) founded the first Italian school of genetics, and became a learned investigator of the biology of Termites and generally in insect physiology. Some students of his school became well reputed geneticists, as did Adriano Buzzati-Traverso (1913-1983), Renzo Scossiroli and Riccardo Milani, all working on *Drosophila*. One further member of the Pavia group, Mario Pavan (1918-), became a well known expert in insect speleology, physiology and ecology.

Also Parma had good entomologists. First of all the dipterologist Camillo Rondani (1808-1879), investigator of the biological equilibrium by original experiments of natural control. Subsequently Giovanni Passerini (1816-1893), professor in the

University of Parma, made important studies on the Aphididae. In this line we must put also the great coleopterist Antonio Porta (1874-1971) author of "Fauna Coleopterorum Italica", in 8 volumes, a free lecturer in Parma University.

In Modena the isolated collector Luigi Picaglia (1816-1893), published on the insects of the territory.

A peculiar centre for entomological studies has been Genoa, where already Massimiliano Spinola (1780-1857), hymenopterist, published "Insectorum Liguria species novae aut rariores" (1806-1808). Subsequently Giacomo Doria (1840-1913), a great traveller and naturalist founded in Genoa a "Museo Civico di Storia Naturale" (1867) as the location for the study of all the material that the numerous expeditions had collected in tropical countries around the world. He was a learned entomologist and the first director of the Museum. At that time the professor of Zoology of the University, Corrado Parona (1848-1922) published on Apiculture and on Apterygote insects. In the Museum, after Doria, the famous coleopterist Raffaele Gestro (1845-1936) became director and created a good team of entomology curators, including the traveller Leonardo Fea (1852-1903), the hymenopterist Giacomo Mantero (1878-1949), Luigi Masi chalcidologist who came from Naples, the coleopterist Edoardo Gridelli (1895-1958) from Trieste, the coleopterist and orthopterist Felice Capra (1896-1991), the coleopterist and hymenopterist Armando Baliani (1874-1895), the hymenopterist Delfa Guiglia (1902-1983). Other important fellows of this group were the amateurs Agostino Doderò (1864-1937), coleopterist, Cesare Mancini (1881-1967), hemipterist, Emilio Berio (1905-1993), lepidopterist, and Giovanni Binaghi (1907-1975), coleopterist, as well as Ferdinando Solari (1877-1956), curculionidologist, and Fabio Invrea (1884-1968), specialist in Mutillidae, who both were in turn presidents of the "Società Entomologica Italiana", after having transferred it from Florence to Genoa in 1922.

Like Genoa, also Trieste had a good tradition of entomology in the "Museo Civico di Storia Naturale" of Trieste and in the Museum "Pietro Rossi" of Duino. A line of important coleopterists worked in the two institutions: Arturo Schatzmayr (1880-1950), Giuseppe Muller (1880-1964), Carlo Lona (1885-1971), Edoardo Gridelli (1895-1958) and the myrmecologist Bruno Finzi (1897-1941). Also Fiume had a good entomologist, Guido Depoli (1879-1948) who studied the coleopterans of Liburnia.

In Venice Nicolò Contarini published a catalogue of insects of the area of Venice and Padua (1843) and Alessandro Ninni (1837-1892) did further orthopterological contributions in the same area.

In Vicenza the abbot Francesco Disconzi published in 1865 "Entomologia Vicentina".

A particular attention must be paid to Verona, where already in the eighteenth century Zaccaria Betti (1732-1788) published a poem on the silk worm, two other books on the same type of moth and on the worms of the apple tree. Orseolo Massalongo (1854-1901) published a "Prospetto ragionato degli insetti della provincia di Verona" (1891). Subsequently Edoardo De Betta (1822-1896) published observations on locusts and bees, Adriano Garbini (1857-1940) worked in hydrobiology and apiculture, and Alberto Brasavola-Di Massa (1886-1956) studied the coleopterans of Trento. But it is in the present century that entomology in Verona, at the "Museo Civico di Storia Naturale", reached a world wide reputation under the leadership of Sandro Ruffo (1915-), a famous coleopterist and biogeographer and chief of an appreciated entomological school.

Also Trento had some good entomologists: the Canestrini family, with Giovanni Canestrini (1835-1900), professor in Padua, an appreciated acarologist who wrote also a good treatise

on apiculture, Pietro Buffa (1871-1941), thysanopterologist who worked some years in Padua and in Florence, the Cobelli brothers, Giovanni (1849-1937), director of "Museo Civico" of Rovereto and Ruggero (1838-1921), who wrote on homoptera, orthoptera and hymenoptera of the region, the applied entomologist Agostino Lunardon (1857-1933), and the great investigator of Bombyx Luciano Pigorini (1882-1967).

After the major entomologists of the seventeenth century Aldrovandi and Malpighi, Bologna continued the tradition in the last two centuries with the applied entomologist Giuseppe Bertolani (1804-1878), the coleopterist Andrea Fiori (1854-1933), Carlo Ermery (1848-1925), professor of Zoology, one of the best myrmecologists of any time. After him, his former student Guido Grandi (1886-1970), who spent many years in Portici assisting Filippo Silvestri, covered the first chair of entomology in Bologna University, and founded a famous Institute and an appreciated journal ("Bollettino dell'Istituto di Entomologia dell'Università di Bologna"), and was one of the founders as well as the first president of the "Accademia Nazionale Italiana di Entomologia". Grandi was a great scientist. He studied comparative morphology and ethology in a number of injurious insects, created a new approach to agricultural entomology studying the various biocenoses, was a major taxonomist of Agaonidae, investigated the ethology of Hymenoptera, studied and defended the biologic equilibrium, discouraging the use of insecticides, wrote the fundamental treatise "Introduzione allo studio dell'entomologia". He had a superb circle of students, assistants and associates who obtained prestigious academical positions: first of them the professor of Zoocultures Anita Vecchi (1893-1953), a learned investigator on Apis and Bombyx. Subsequently the already mentioned Athos Goidanich who founded the Entomology Institute of Turin, the hemipterist Antonio Servadei (1908-1979), who had previously started a valid school in Sassari, and later in Padua, the dipterist Filippo Venturi (1910-1975) founder of a good school in Pisa, the ethologist Leo Pardi (1915-1990), who started the Italian comportamental ecology and had valid students and associates in Florence, the aphidologist Minos Martelli, who succeeded to Grandori in Milan, the already mentioned coleopterist Sandro Ruffo, who started the school of Verona, the neuropterist Maria Matilde Principi who succeeded to Grandi's chair, the coleopterist Giorgio Fiori (1923-1983), the tachinologist Egidio Mellini, the forest entomologist Rodolfo Zocchi, who directed, following Melis, the "Stazione di Entomologia Agraria" in Florence, and many others including the lepidopterist amateur Attilio Fiori (1883-1958), the myrmecologist Carlo Menozzi (1892-1943) and the ephemeropterist Marta Grandi (1915-). The already mentioned Giorgio Fiori started good schools in Sardinia first and later in Perugia, where the trichopterologist Giampaolo Moretti and his school in the Zoology Institute were also working.

In Palermo there was the already mentioned Paolo Boccone, who published some entomological observations and studied apiculture. This city too, had good entomologists in the eighteenth century: the agricultural entomologists Francesco Minà-Palumbo (1814-1899) and Teodosio De Stefani-Perez (1853-1935), the taxonomists Giuseppe Riggio (1848-1914), Enrico Ragusa (1849-1924), Luigi Failla Tedaldi (1853-1930), the cytologist and embryologist Andrea Giardina (1875-1948), professor of zoology, the lepidopterist and medical entomologist Mario Mariani (1898-1965), and has now an active University Institute of Agricultural Entomology.

A peculiar situation, which was left last in this overview, is the one regarding the Roman Entomology. As we have seen the imperial Rome had the pride to host several of the Latin encyclo-

pedic entomologists. Unfortunately very few scientific works were carried out here until the end of the nineteenth century, except for a few isolate works, as far the already mentioned microscopists Francesco Stelluti and Filippo Buonanni, or the applied orthopterists Luigi Doria, who published in 1816, and Luigi Metaxà (1778-1842), who published in 1825, or Carlo Luciano Bonaparte (1803-1857), who did some researches on lepidoptera. In the second half of the nineteenth century Giovanni Battista Grassi (1854-1925), covered the chair of Comparative Anatomy at the University and started a series of outstanding entomological researches. Initially he studied the taxonomy and embriology of the honey bee, but later he switched on to the comparative morphology of Myriapoda and Thysanura ("I progenitori degli Insetti"), and to the sociality of Isoptera ("Costituzione e sviluppo della società dei Termitidi"), of the Embioptera, and their intestinal symbionts. In 1896 the pathologist of Rome University Amico Bignami (1862-1929) suspected that the malaria was inoculated by mosquitoes. Immediately Grassi devoted himself to the malaria problem revealing, in 1898, the importance of Anopheles (while Roth, in the same year spoke about "some mosquitoes") in the transmission of the disease, following in the cells of the body of the insect the development of Plasmodium ("Studi di uno zoologo sulla malaria", 1900). After a study on Phlebotominae, he discovered the complex cycle of Phylloxera, and published the book "Contributo alla conoscenza delle Fillosserine ed in particolare della Fillossera della vite" (1912). It is clear that this variety of investigations could have been enough to assure the glory to four or five scientists. The phenomenon of Grassi is destined to remain unrivalled. Obviously he had a lot of students too: among the entomologists we find the already mentioned Silvestri, Anna Foà (1876-1944) who covered the chair of sericulture in Portici, the already mentioned Carlo Jucci, founder of Italian entomological genetics in Pavia, and Remo Grandori, the

founder of the modern school of entomology in Milan. For these years, around the professor of Zoology Antonio Carruccio, a modest museologist, former pupil of Adolfo Targioni Tozzetti, we find in Rome only passionate and learned amateurs: a line of coleopterists, Paolo Luigioni (1873-1937), author of "I coleotteri d'Italia", Pio Mingazzini, Adelchi Tirelli, G.E. and F. Rasetti, Marcello Cerruti (1818-1870), a line of lepidopterists, Fortunato Rostagno (1853-1934), Lillo Barbera, Sergio Beer, Orazio Querci, Enzo Romei, Federico Hartig, a good line of travellers and tropical collectors, as Vito Zanon (1875-1949), as the great speleologist and traveller, Saverio Patrizi-Montoro (1902-1957) and the more famous traveller, Edoardo Zavattari (1883-1972) who in turn directed the Institute of Zoology. Moreover we have the dipterist Giuseppe Tuccimei (1851-1915), the hymenopterist Giuseppe Lepri (1870-1952), and a line of parasitologists, as Giulio Alessandrini (1866-1954) and Giuseppe Saccà. Grassi's had been for all of them an outstanding personality. After his death, entomology in Rome was neglected, and almost half a century passed before the signs of a renaissance could be seen. This happened thank to the work of an obscure primary school teacher, Omero Castellani (1903-1974), a modest, but passionate collector, who directed a lot of children to work with himself. He founded in 1945 the ARDE (Associazione Romana di Entomologia), publishing a small Bulletin. The children grew up and became the well reputed professors of Entomology and of Zoology in several Universities of Rome and neighbouring cities.

Italian entomology is a strong tree. This congress has the honour to host representatives of the many branches of the schools related to Targioni, Costa, Emery, Berlese, Silvestri, Grandi, Jucci, Goidanich, Ruffo, La Greca and the other protagonists of this history. I hope you will look on them, and unveil through in their personalities the traces of their long ancestry.

INSECTS: THE ULTIMATELY BIODIVERSE ANIMALS

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Viewed as part of the great evolutionary tableau of life, the most distinctive features of insects is their dominance on the land, which has persisted in stark contrast for 360 million years with their virtual absence in the sea. They are the most numerous animals of intermediate size, that is, 0.1 mm - 100 mm in length, and possess the greatest total biomass of any terrestrial class or phylum of animals. Insects are also hyperdiverse (Wilson, 1992). They fit one of the rules of biodiversity: within each monophyletic assemblage of species (such as a family) it is typically the case that one of the constituent taxa (a genus in the family) is far more diverse (in this case comprises more species) than can be expected by chance alone (Dial and Marzluff, 1989). The assemblage, in other words, is off the curve, significantly higher in representation than would be expected if it belonged to an ordinary, randomly generated frequency distribution. The most hyperdiverse taxa, in descending taxonomic rank from kingdom downward are: Arthropoda within the Animal Kingdom, Hexapoda (insects) within the Arthropoda, Coleoptera (Beetles) within the Hexapoda, Chrysomelidae (leaf beetles) and Curculionidae (weevils) within the Coleoptera.

How many species of insects are there? Between 750,000 and 1 million nomenclaturally valid species have been described; the exact number is not known because no catalog of the entire world fauna exists at this time. Also, the percentage of synonyms, possibly as high as 10 percent, is unknown. An intermediate number in this range, say 800,000, composes very roughly three-quarters of all animal species and half of all species of all kinds of organisms.

The *true* number of insect species, described plus undescribed, can only be guessed to the nearest order of magnitude. Extrapolating upward from the number of beetle species in the canopy of a single tiliaceous tree in Panama, Terry Erwin (1982, 1983) famously estimated 30 million insect species worldwide. Although his pioneering effort spurred worldwide interest in the great magnitude of insect diversity, there were potential large errors in each step of the extrapolation-including variation in arboreal residents from one tree species to the next, the percentage specialized on each tree species, localness of the specialized species, and others-to move the permissible estimate up or down several fold. Estimates by other authors based on extrapolation upward from entire well-censused faunas, including the Hemiptera of part of Sulawesi and the insects of great Britain,

place the number of insect species worldwide at 2-6 million (review by May, 1992). Even this more modest diversity, if correct, far outweighs that likely to exist in all the rest of the Animal Kingdom.

Why are there so many insect species? We have only hypotheses, and even taken together they still might not identify all the parameters. One key factor is surely preemptive antiquity: the insects were able to saturate terrestrial and freshwater habitats very early in geological history of life on the land. Apterygotes appeared by the middle Devonian, where they coexisted with centipedes, millipedes, spiders, and trigonotarbids, and winged insects by the end of the Lower Carboniferous (Gray and Shear, 1992). During the Upper Carboniferous and Permian, there followed a massive adaptive radiation, peaking at 30 orders, close to the number today. The number of families rose more or less steadily throughout this history, with a dramatic jump during the Cenozoic in concert with the expansion of the flowering plants (Carpenter, 1992; Jarzembowski and Ross, 1993).

The evidence suggests that the preemption entailed early and multifarious adaptations to terrestrial vascular vegetation, from its earliest origins to the present day. Many of the most diagnostic characters of insects, including the details of metamorphosis, feeding devices and physiology, nest-building, and oviposition behavior, reflect fine-tuned exploitation of plants, as well as (especially for saprophages and carnivores) life in the soil.

The quantum advances in the evolutionary history of insects were first their origin as a fully terrestrial group; second, the invention of adult winged flight, the first such achievement in the history of life; third, the folding of wings, allowing penetration of winged adults into small spaces; fourth, the origin of advanced hemimetabolous and then holometabolous metamorphosis, which in conjunction with flight allowed exploitation by individual species of a wider range of niches; and finally, the origin of the social insects, whose superbly integrated colonies came to dominate much of the land environment during the Cenozoic era.

Insect diversity is one of the most important but least understood phenomena in science today. It has profound implications for our understanding of evolution and ecology. Furthermore, given the ubiquity and power of insect life all around us, its careful study is necessary for the planning of a healthy, sustainable human environment.

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COMPARATIVE SPERMATOLOGY IN INSECT TAXONOMY AND PHYLOGENY

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In a previous paper¹ I have reviewed the fantastic differentiation and evolution of sperm structure and function in the Insecta, and I have observed that the various organelles typical of insect groups tend to persist in advanced levels of evolution and differentiation. This is in agreement with the principle of perseverance in evolution proposed by Baccetti².

I will now present a general picture of insect sperm features, ordering the various findings into a phylogenetic tree (Fig. 1). Phylogenetical insect spermatology was attempted by Retzius³ and Franzen⁴ at the light microscopy level, by me in several subsequent papers^{1,5-8} and by Dallai⁹ based upon electron microscopy. Jamieson¹⁰ recently produced a comprehensive treatise on the subject. In this paper I reduce to a minimum the references: the full literature is presented in the papers 2-10, and particularly in 1.

As observed by Baccetti¹, the sperm model closest to that of the presumed Arthropod ancestor is retained (Fig. 1) by the encysted Collembola sperm and by the free sperm of iapiigid Diplura (acrosomal complex endowed with perforatorium, 9+2 axoneme, conventional mitochondria). In campodeid diplurans the typical insect axoneme pattern, 9+9+2, appears. Here the accessory microtubules group in one bundle and retain the primitive number of 13 protofilaments in the cross section of their wall. From this model the Archeognatha sperm seems to derive; they show, in fact, accessory tubules grouped in two rows and containing 16 protofilaments in cross section of their wall. The Zygentoma model is more advanced, and closer to that of most Pterygota, having the 9+9+2 axonemal pattern with accessory tubules comprised of 16 protofilaments, each positioned near one doublet. But a parallel, independent line, that of Protura, seems to have started from the Insect ancestor. Here we see two evolutionary steps. The first step belongs to genera with encysted sperm, which are motile only when extremely close to the egg in the female genital apparatus, the second belongs to definitely immotile genera. None of them exhibit evolution toward the classical insect sperm characters. In the encysted, elongate model (*Acerentomon*, *Acerentulus*, *Eosentomon* a.o.) the acrosomal complex lacks a perforatorium, mitochondria are still conventional, and the axoneme is comprised of doublets endowed with only the inner dynein arm and arranged according the 12+0, 14+0 or 16+0 pattern. In the immotile model the regression is still more evident: the sperm is globular or discoidal, devoid of acrosomal complex, centriole, axoneme, and comprised of only a nucleus and a few conventional mitochondria.

In this long path of step by step regression, Protura recall more strictly Pycnogonida or Arachnida than Insecta.

The sperm model of Zygentoma leads into Pterygote insect phylogeny. All groups seem to be clearly characterized by hyper-telic sperm, and by the 9+9+2 axonemal pattern with 16 protofilaments forming the accessory microtubule wall. The only exception are Ephemeroptera (the oldest Pterygota), where the classic

insect sperm is still lacking, the acrosomal complex is monolayered (perforatorium absent), the axoneme is 9+9+0, doublets have only the inner arm and accessory microtubules have the primitive number of 13 protofilaments in their wall. Moreover, the single mitochondrial derivative has conventional cristae, no crystallomitin, and is flanked by two crystalline rods which play the role of accessory bodies. The highly evolved ephemeropteran family Leptophlebiidae has coccoidal sperm containing a perforatorium in the center of the acrosome, and one conventional mitochondrion or no mitochondria at all, according to the species. Centriole and axoneme are absent. The first are primitive characters, aflagellarity is a quite specialized one. This complex picture indicates Ephemeroptera as a very peculiar and independent branch among pterygote insects, conserving some plesiomorphic characters lost elsewhere but still evolved toward immotility. The first, real pterygote sperm is present in Odonata. They have a bilayered acrosome, a 9+9+2 classic axoneme with 16 protofilaments in the wall of accessory tubules, two partially crystallized mitochondrial derivatives (crystallomitin protein synthesis starts at this level) and two compact accessory bodies flanking the axoneme. No involved or aflagellate forms are present in this order, which exhibits the most classic insect sperm model. From several points of view Ephemeroptera and Odonata seem to be absolutely independent orders, and the group Palaeoptera appears to be a quite artificial taxon (Fig. 1).

All other orders belong to the Neoptera branch, and can be grouped in the three classic divisions of Polyneoptera, Paraneoptera and Oligoneoptera (holometabolic insects) (Fig. 1).

As sperm structure is concerned, all the orders of Polyneoptera (Fig. 2) except Isoptera and Phasmida appear quite similar, retaining almost all the characteristics of the pterygote sperm model. Isoptera and Phasmida, in fact, show very peculiar sperm characters that suggest a long and quite independent evolution.

In the more traditional stock the most conservative sperm model is that of Blattodea having a long sperm (some hundreds μm), with an acrosome endowed with a perforatorium, a 9+9+2 axoneme, having 16 protofilaments in the accessory tubules, flanked by two long, partially crystallized mitochondrial derivatives and more or less long dense processes arising from the centriolar adjunct. From the adjunct delicate links (connecting bands) also arise joining the mitochondrial derivatives to the axoneme for almost their entire length. One long, flat, membranous cistern is present close to the adaxonemal surface of each derivative. Mantodea maintain all of the blattoid characters, but have a quite short perforatorium, and the system of links between mitochondrial derivatives and axoneme is much richer, including two dense lateral and one or two additional delicate central bands. From this ancestral dictyopteran stock five main phylogenetic lines seem to have radiated. The Grylloblattodea line is peculiar. It retains all the characters of Blattodea, but has

only a monolayered acrosome, and therefore appears more evolutionarily derived. A second line includes Dermaptera, Embioptera and Plecoptera. They retain blattoid axonemal and periaxonemal structures and the partially crystallized mitochondrial derivatives, but while Dermaptera have a 3-layered acrosomal complex (plesiomorphic) the other two orders have a monolayered one, because only the acrosome is present, and therefore they seem to be more derived. All the three orders have more or less long processes arising from the centriolar adjunct. However, while Dermaptera have a single double triangular process close to the axoneme, Plecoptera have a long uneven, lateral rodlet flanking the axoneme and Embioptera have three rodlets: a bilateral pair of long rodlets, and one shorter rodlet.

The three other lines each end with one of the three classic orthopteran superfamilies Tettigonoidea, Grylloidea (suborder Ensifera) and the suborder Caelifera (Fig. 2). An evident preliminary step in the line to Tettigonoidea is the strange Australian group of Coloolidea which exhibits all the characters of the dictyopteran stock, including the three connecting bands, but has a flat nucleus in a flat head. The last in Tettigonoidea becomes arrow-like because of the peculiar shape of the acrosomal complex and because an accessory extra-axonemal longitudinal microtubule appears between each of the lateral connecting bands and the axoneme. Accessory bodies are absent. On the line going to Grylloidea, Gryllacridoidea are inserted. This group retains all the blattoid characters, and in addition acquires two accessory bodies that in some species are very small, no larger than a microtubule, while in others are bigger, and crescent shaped. Grylloidea exhibit the same characters, but in the place of accessory normal microtubules or bodies have two 10 nm thin tubules, and in most families a long segment of the terminal part of the axoneme is stiffened and immotile due to tanning of doublet and singlet microtubules. While the examined orthopteran superfamilies (all ensiferans) have in common only basic features of dictyopteran stock, Caelifera show some important, new characteristics which render the group homogeneous. The most evident is that the sperm tends to be extremely thin, elongate-cylindrical and as a consequence the extra-axonemal cytoskeleton increases, as well as the two mitochondrial derivatives that are almost completely filled with crystallomitin, and have the transversally oriented cristae restricted to a reduced, thin cortex. Moreover, a pair of thin, extra-axonemal 10 nm thin tubules flanks the axoneme laterally in almost all the superfamilies. Furthermore, the acrosome is always inserted on the nucleus by two lateral processes that flank the nucleus itself on two sides. The various superfamilies progressively show further characters. Cyndrachetidae have a rather primitive sperm that is still similar to the dictyopteran sperm (obviously with the addition of the three general caeliferan characters listed above). It has, in fact, a three-layered acrosome and 9+9+2 axoneme, but has 3 connecting bands, the two lateral containing one extra-axonemal 10 nm thin microtubule. From this model, assumed as ancestral for all caeliferans, one or two lines arise. The first one includes Tetrigoidea and Tridactyloidea, which are very similar each to the other, exhibiting all the cyndrachetan characters. In addition, the two mitochondrial derivatives differ in size and generally the number of connecting bands is greater than two. In Tetrigoidea there are 2-4 of these bands, and the two extra-axonemal tubules reach the dimensions of normal microtubules; in Tridactyloidea there are five bands, and in the place of the extra-axonemal microtubules two dense, accessory bodies are present. They recall the two crystalline accessory bodies typical of Phasmoidea, and, like them, are derived from the activity of the Golgi complex.

The second line includes the Eumastacoidea and the Acridomorpha. All the cyndrachetoid characters are conserved; the two mitochondrial derivatives are equal in size and shape. An important new character is that the entire plasma membrane (except in the very tip of the acrosomal region) is covered by a thick glycocalyx forming a fuzzy coat of 10 nm or more-long microfilaments arranged perpendicular to the surface. Eumastacoidea have three evident granular connecting bands. The two lateral bands contain an extra-axonemal 70 nm microtubule. In this group the microfilaments of the fuzzy coat are 10 nm long. Acridomorpha have two thin connecting bands between derivatives and axoneme. The bands contain a thin, 10 nm microtubule. The centriolar adjunct is semicircular in section posteriorly. It is dense, and partially embraces the beginning of the axoneme and is structured in several thick longitudinal elements that are rods or laminae, according to the family. The microfilaments of the fuzzy coat are 30 nm long.

As we have previously observed, Isoptera show, in the various families, every kind of variation. First, none retains the conventional spermatozoon. The acrosome is consistently absent. The unique model of axoneme is that of the multiflagellate sperm of Mastotermitidae, and it is quite aberrant e.g., 9+0 without outer dynein arms. Moreover, few conventional mitochondria are present, and no accessory bodies. All the other, higher isopteran families have also lost the axoneme, and have aflagellate immotile sperm comprised simply of the nucleus and a few mitochondria. But Calotermitidae have evolved further and have several outpocketings of the plasma membrane that contain a rich cytoskeleton of microtubules. This group seems to be progressing toward a new motility. Evidently Isoptera separated from the blattoidean stock quite early, and have followed a long, drastic and independent evolution, attaining a series of synapomorphies unique in the Animal Kingdom.

Phasmoidea are in some ways more conventional, and certainly more homogeneous in sperm structure. Nevertheless the complex of synapomorphic characters is impressive: an acrosomal complex without a perforatorium, a lack of mitochondria, and consequently a peculiar axonemal metabolism and 17 protofilaments in the accessory tubules, enormous accessory bodies comprised of cytokeratin. In comparison with all the orders grouped in the classic blattoid-orthopteroid stock they are certainly the most highly evolved (Fig. 2).

In the paraneopteran orders sperm evolution seems to be even more dramatic but identification of the direction of the series of modifications is easier (Fig. 3).

Psocoptera are the first order of the group. They have a monolayered acrosomal complex, centrioles formed only of doublets, a 9+9+2 axoneme (one species with 13 protofilaments in the wall of accessory tubules) and two crystallized mitochondrial derivatives. The most primitive species have the usual single axoneme while others are more or less constantly biaxonemal. In fact the species belonging to Procomomorpha have almost all monoflagellate spermatozoa, while Trogomomorpha have one-fourth of the sperm in the ejaculate exhibiting two axonemes, and Troctomorpha have only biaxonemal spermatozoa. Mallophaga and Anoplura, orders that form with Psocoptera the homogeneous group of psocoid insects, have constantly biaxonemal spermatozoa, similar to those of the higher psocopterans. After these orders, where the biaxonemal condition seems to be affirmed, evolution follows two diverging branches. One of them concerns Thysanoptera, which reach a condition of three axonemes, arisen from three centrioles, mixed, disordered and incomplete. The acrosomal complex lacks a perforatorium. A single mitochondrial derivative with normal shape and crystal-

lization is present; accessory bodies are absent. Even if the sperm is motile, this organization suggests a tendency toward immotility. The second branch (Fig. 3) encompasses the order Hemiptera. Already in the suborder Heteroptera, Hemipterans show an important evolutionary progression with respect to the psocoid orders. First, the main pterygote characteristic of hyper-tely is accentuated (some species contain spermatozoa that are 5 mm in length, but are only 1 μ m wide). Therefore, the cytoskeleton of the sperm is drastically evolved. The acrosome, that can be very long, up to 2.5 mm, contains not only a strong actinic perforatorium, but also bundles of narrow microtubules. The axoneme, drastically 9+9+2, has accessory tubules that with respect to those of psocoids are larger and consist not of 13 protofilaments, but of 16, a number largely present among pterygotes. The two mitochondrial derivatives are enormous, strongly crystallized and contain at least three patterns of crystallization of the crystallomitin. As in polyneopterans, each mitochondrial derivative is linked to the axoneme by a delicate bridge. The retained psocoid characteristic is a tendency toward bi- or multi-flagellarity, that in some species seems to be constant, with one longer and one shorter axoneme, and toward the absence of accessory bodies.

In the suborder Homoptera (Fig. 3) sperm evolution reaches one of its highest expressions in the Animal Kingdom. In Auchenorrhyncha most of the heteropteran characters are retained. Important acquisitions are that the acrosomal complex appears devoid of a perforatorium and other cytoskeletal devices, large and peculiar accessory bodies are present and the tip of the tail is frequently branched. The shape of the accessory bodies offers excellent diagnostic characters reaching the specific level. The lower families of Sternorrhyncha continue showing the same general pattern as Auchenorrhyncha, even if Psylloidea have peripheral arborescent appendages that seem to have the same origin as the acrosome. The same appendages are present in the subsequent infraorder Aleyroidea whose mature sperm is immotile, lacks a true acrosome and axoneme and has two mitochondrial derivatives reduced to a pair of small granular bodies exhibiting no cytochrome-C-oxidase activity. The spermatid contains a 9+9+0 axoneme, a cap-shaped acrosome and normal mitochondria that are reduced or disappear at the end of the regressive spermiogenesis. Coccoidea are the last hemipteran infraorder. Their sperm is still devoid of an acrosome, mitochondria, centrioles and axoneme, and has regained motility by using the spermatid microtubular manchette, which is now transformed into a system of perinuclear parallel singlet tubules endowed with a series of single arms containing a MAP (microtubule associated) protein. Throughout Paraneoptera the possible evolutionary pathway of the sperm seems therefore to be complete. It extends from the classic typical pterigote sperm, to the biflagellate sperm, to the disordered axoneme with reduced motility, or to the hypertelic axoneme, followed by axoneme involution and loss, and finally by regained motility utilizing new devices.

Does a similar evolution occur in the enormous group of Oligoneoptera (holometabolic) insects? Here the phylogeny is less clear, and the general picture of the spermatozoon is more uniform. Nevertheless, interesting lines of evolution can be envisaged (Figs. 4-6). If we look for the presence of plesiomorphic characters, we see that oligoneopterans start with patterns similar to those of the lower forms of Apterygota and do not represent a further step after Para- or Polyneoptera (Fig. 1). In fact, we find examples of 9+2 axonemes, three-layered acrosomes and even biaxonemal forms that are close, therefore, to the very origins of terrestrial sperm evolution. The presence of such models has been sometimes interpreted as an example of highly

derived, regressive evolution, but given their frequency in a particular branch of oligoneapteran phylogeny, it also deserves to be considered as reflecting a basal position, without further speculations (Fig. 1).

The first group to be considered is the "panorpid orders", a group recognized by almost all insect phylogenists, and demonstrated only on spermatological basis by Baccetti⁶. These orders are Mecoptera, Aphaniptera, Diptera, Trichoptera and Lepidoptera (Figs. 1, 4, 5). In Mecoptera, Aphaniptera, primitive Diptera (the mycetophilid *Keroplatus*) and a few Trichoptera the axoneme is 9+2 and the mitochondrial derivatives are almost completely crystallized. The first character is an indication of plesiomorphic condition, and would indicate an ancestor of the group in the dipluran stock; the second indicates a more derived state. It places the origin of the group after the acquisition of crystallomitin, at least after the Zygaentoma branch off, and certainly after the branching of Palaeoptera, which are devoid of crystallized derivatives. I do not believe that the "9+2" of primitive panorpoids is a regression that occurred in their ancestry (i.e., an apomorphy) immediately after the long and complex acquisition of the "9+9+2" which promptly reappears in all the same panorpid orders with a clear synapomorphic process. It is rather possible that crystallomitin ancestor was already present during dipluran evolution together with the nine accessory tubules in the axoneme and that these characters were acquired and evolved in all lines derived from them, even though these were not affirmed in Palaeognatha. In fact, we have seen in most phyletic trends of Pterygota many instances of lost crystallization in mitochondria, usually occurring along with phenomena of axonemal regression.

Having noted the above, in panorpid evolution, both Mecoptera and Aphaniptera (Fig. 1) are "9+2" and have crystallized mitochondrial derivatives (one, crescent shaped in section, in the first order; two, one larger and cylindrical and the other smaller, in the second). The centriolar adjunct is present, even if short and caudally extended in a short process in some species. In Aphaniptera, moreover, two crystalline accessory bodies are extended for 500 μ m flanking the axoneme. The Diptera are another parallel branch arising from the panorpid tree (Fig. 4). There the Mycetophilidae family offers the key to the enigma (Fig. 4), having some species (*Keroplatus*) with a 9+2 axoneme as in Mecoptera and Aphaniptera. Only one partially crystallized mitochondrial derivative is present, the acrosome is monolayered and is devoid of a perforatorium, and accessory bodies are absent as in all Diptera. The whole family retains their character, but an important synapomorphy appears. The set of nine accessory axonemal tubules is progressively developed: they are zero in *Keroplatus*, two in *Tarnania*, seven in *Exechia*, nine in *Mycetophila* and *Leptomorphus*. Moreover, the wall of these singlets contains in section 15 protofilaments in some species and 16 in others. This observation substantiates the view that the mycetophilid tail retains plesiomorphic characters (and therefore the 9+2 is plesiomorphic) and that progressive acquisition of the 16 protofilaments is the most generalized, synapomorphic model in pterygotes. It would not be surprising to find some primitive mycetophilid species with 13 protofilaments (still retained by Tipulidae and Trichoceridae). Even in mycetophilids one species (the "fungus gnat" photographed by Phillips,¹¹) develops a 9+3 axoneme. From the Mycetophilidae line, after the definitive acquisition of the 9+9+2 number, but largely before the acquisition of the definitive 16 protofilaments in the wall of accessory tubules, a group of families branches off (Fig. 4). These families were suspected to be the most primitive of the order, but evidently the lower fungus gnats are even more so. These 9+9+2 fami-

lies are Tipulidac, Trichoceridae, Chironomidae, and Dixidae. The first two have 13 protofilaments in the accessory tubule wall; the latter two have 15. They have apparently branched off at different points of mycetophilid evolution. All have the classic dipteran sperm with the acrosome lacking a perforatorium, two largely crystallized mitochondrial derivatives and no accessory bodies. Other nematoceran Diptera lines have arisen from the mycetophilid stock. Sciaridae have the well known motile sperm with a monolayered acrosome and a single mitochondrial derivative. A giant centriole, gives rise to an axoneme comprised of approximately one hundred longitudinally arranged doublets with two dynein arms. Each doublet is flanked by an accessory singlet tubule having 13 protofilaments in the wall. It is possible that this peculiar line branched early from the mycetophilid line, together with the Tipulidae and Trichoceridae families which retain the 13 protofilaments in the wall of accessory singlets. Another line arisen very early from the basic stock is the Psychodidae. Here the more primitive Phlebotominae are evolving towards immotility, having a 9+9+0 axoneme and a single partially crystallized mitochondrial derivative, while the Psychodinae exhibit the described regressed aflagellate model with only one normal mitochondrion and a large acrosome. The most peculiar characteristic is that Phlebotomus has a recognizable subacrosomal perforatorium (the only example of a bilayered acrosome among dipterans). This, together with the pattern of regressive terminating in aflagellarity, suggests that Psychodidae branched out from ancestral dipterans before or at the beginning of mycetophilid evolution. Other Nematoceran families, branched off later from the mycetophilid stock, are evolving toward immotility. All have a monolayered acrosome and, at least in those examined so far, 15 protofilaments in the wall of the accessory single tubules. But while Bibionidae have a 9+0, or 9+9+0 axoneme (one empty central cylinder is present) and one well-crystallized mitochondrial derivative, Culicidae always have a 9+9+1 central rod in the axoneme and two derivatives while Simuliidae have a 9+9+3 axoneme and one derivative. Also, two crescent-shaped accessory bodies are present.

But the most spectacular evolution occurs in cecidomyid dipterans (Fig. 4). As noted above in the Cecidomyidae a primary step is the presence of one or two 9+0 axonemes lacking of dynein arms. In this category only the genus *Myricomyia* has one immotile 14+0 axoneme with armless doublets, and the species *Contarinia flori-perda* has an immotile 12+0 axoneme made up of armless doublets. All these taxa belong to the supertribes Oligotrophidi, Lasiopteridi and part of the Cecidomyidi. Around the axoneme (or axonemes) more or less numerous single microtubules which were part of the perinuclear manchette of the spermatid, are present. One species (see above) of the genus *Lestodiplosis* (also belonging to the Cecidomyidi) has a sperm (obviously immotile) completely lacking an axoneme and exhibiting single tubules. Therefore, with the total lack of an axoneme, immotility has been achieved. However, cecidomyid evolution progresses further. Many genera of the highest Cecidomyidi and the Asphonodyliidi have motile sperm where the manchette increases in the number of microtubules. The microtubules assemble two by two, forming doublets. Each doublet bears only a series of arms (apparently the outer) endowed with a single MAP (dynein) band. In the family therefore there is initially a regression, followed in *Lestodiplosis* by the complete disappearance of the classic motility apparatus (the two armed doublet), and finally the progressive reacquisition of a similar device by a different means, not involving the presence of a centriole.

Compared with the fantastic evolution and variation of Nematocera, dipterans belonging to the suborder Brachycera appear quite uniform. All have extremely long spermatozoa, from some hundred μm to a few mm (up to 6.64 mm in *Drosophila hydei*). The acrosome is monolayered and lacks a perforatorium, as in almost all dipterans. The plasma membrane is asymmetric, having an outermost layer 5 nm thick. There are two well-crystallized mitochondrial derivatives. Sometimes, as in *Drosophila melanogaster*, the derivatives are quite different in size, one of them being extremely small and short. The axoneme is 9+9+2, with two arms in the doublets, and 13 protofilaments in the wall of the accessory tubules. There is an abundant and dense intertubule material, including 13 protofilaments, and double spoke heads. These characters are very similar to those of trichocerid and tipulid nematocerans. Brachycera could have branched from the nematoceran stock at that level (Fig. 4).

Trichoptera and Lepidoptera are two related orders that also belong to the panorpoid group (Fig. 5). As proposed by Baccetti⁷, their sperm structure suggests that they could have arisen from a mecopteran-like ancestor. These two orders have many characters in common, first of all the heterogamety of the female sex. Large axonemal accessory tubules, filled with a glycidic material also are a characteristic common to the two groups. Trichoptera (Fig. 5) are usually assumed to be a less derived group, and their sperm structure substantiates this view. The suborder Annulipalpia is characterized by non-crystallized mitochondria, that are frequently conventional (or absent), by a monolayered (or absent) acrosome, and by the lack of accessory bodies. The axoneme shows the entire series of transformations already found in other groups with long and multiform evolution. A few species have the basic 9+2 axoneme, for example, a primitive hydropsychid, *Macronema*; others belonging to the genus *Polycentropus* have the 9+9+2 or 9+9+3 pattern, all with doublets having only the inner dynein arm. Other *Polycentropus* and *Hydropsyche* species have the strange 9+7 pattern, with immotile doublets lacking arms. Other Hydropsychinae have an aberrant immotile spermatozoon with no acrosome. The nucleus is lateral and several (more than 9) armless doublets arise from the basal body and individually protrude as single digitations from the cell body¹². Finally, aflagellarity is reached in the philopotaminid *Chimarra florida*, where the mature spermatozoon contains only elongate, horseshoe and apically annular in cross section nuclei, while the acrosomal complex and mitochondria are lacking. Conversely the suborder Integripalpia exhibits more conventional spermatozoa having a 9+9+2 axonemal pattern with monoarmed doublets, one mitochondrial derivative (always lacking crystalline material), and a monolayered, or absent at all, acrosomal complex. Dallai and Afzelius¹³ investigated the number of protofilaments in the accessory tubules, finding the basic number of 19, but variation from 19 to 16 along the length of each tubule. It seems therefore that Annulipalpia exhibit the entire range of character states (from 9+2 to aflagellarity) while Integripalpia have branched from the Annulipalpia line before regression of the motility apparatus, conserving the 9+9+2 constant structure. The similarity with nematoceran and brachyceran evolution is evident.

Lepidoptera sperm (Fig. 5) resemble trichopteran sperm in having large, more than 30 nm thick accessory tubules (with 16 protofilaments in their wall) in a conservative 9+9+2 axoneme. The acrosome is extremely reduced, monolayered and frequently absent. There are usually two mitochondrial derivatives that are partially crystallized, cristate, and unequal in size. Accessory bodies are absent. All Lepidoptera (including Zeugloptera and Monotrysia) have two kinds of spermatozoa: eupyrene spermato-

zoa that have a nucleus and are fertile; and the apyrene spermatozoa that lack a nucleus but play an essential although enigmatic role in the fertilizing ability of eupyrene sperm. Ditrysia are characterized with respect to the more primitive groups by having the glycocalyx of the eupyrene spermatozoa endowed with ordered, crystalline, laciniate or reticulate appendages. Therefore it is very clear that Lepidoptera branched from the trichopteran stock, conserving many trichopteran characters but immediately showing sperm dimorphism. Subsequently, ditrysians branched from monotrystsians, acquiring the laciniate glycocalyx. No evolution towards immotility and aflagellarity seems to have occurred.

The other important group of Oligoneoptera comprises the neuropteroid orders (Fig. 6). The most primitive order is Megaloptera (Fig. 6), showing many characters typical of highly derived Pterygota sperm, and found in Polyncoptera and Parancoptera i.e., three-layered acrosomal complex, 9+9+2 axoneme with two-armed doublets and 16 protofilaments in the wall of accessory tubules, two crystallized, large mitochondrial derivatives, and two accessory bodies. Raphidioptera (Fig. 6), the second neuropteroid order, contains a bilayered acrosomal complex and lacks the extra-axonemal layer. Other raphidiopteran characters are similar to those of Megaloptera. Neuroptera (Fig. 6) are quite similar to the two other orders: the acrosomal complex is very unstable, varying from the three-layered model of *Myrmeleon* to the complete absence of one in *Chrysopa*. The two mitochondrial derivatives are sometimes similar, but sometimes are of different sizes.

This classical sperm model also seems to be present in the other neuropteroid order, Strepsiptera (Fig. 6). Here the axoneme has the 9+9+2 pattern with biarmed doublets and two mitochondrial derivatives, partially crystallized and rich in cristae. The acrosomal complex is monolayered and there are no accessory bodies. Strepsiptera, for these reasons, seems to be a rather homogeneous group, and to have an independent position in the neuropteroid phylogeny.

Coleoptera (Fig. 6) maintain most of the typical neuropteroid characters but, as expected in such a large and diversified order, also show the signs of a long evolutionary history. They branch from the neuropteroid line, retaining a typical acrosomal complex comprised of acrosome and perforatorium (sometimes an extra-acrosomal layer is present), a 9+9+2 axoneme (biarmed doublets and 16 protofilaments in the accessory tubule walls), and two largely crystallized mitochondrial derivatives with cristae localized at the periphery. This structure is, e.g., present in adephagid Cicindelidae, in Carabidae, in the paired sperm of Ditiscidae where the sperm are accompanied by two crystalline accessory bodies and a single perflagellar body derived from the centriolar adjunct. Sperm structure in Cicindelidae is more or less conserved in polyphagid Scarabeiformia and in Bostrychiformia, all of them devoid of accessory bodies and a perflagellar body. But the Staphiliniformia exhibit a level of evolutionary diversity that is exceptional among Coleoptera: Staphilinidae supplement the basic organelles with a perflagellar body, originating from the centriolar adjunct, and two accessory bodies that have more or less the same tail structure seen in the Ditiscidae; Silphidae lack accessory bodies and retain the perflagellar body; the same occurs in the ptiliid *Ptinella* (1.4 mm long spermatozoon!) and probably in *Bambara*, where organelles are arranged in a complicated coiled structure. But in the same family, *Acroticus* is aflagellate, and retains mitochondria and a three-layered acrosomal complex, and *Actinopteryx* in addition, lacks a perforatorium and mitochondria.

A peculiar evolutionary progression also has occurred in the coleopteran group Cucujiformia. This group starts with Cleroidea

that has a three-layered acrosomal complex, a 9+9+2 axoneme, no perflagellar body, two crystallized mitochondrial derivatives and two accessory bodies. Cucujoidea have these same characters, but the acrosomal complex lacks the extra-acrosomal layer, and the two mitochondrial derivatives sometimes differ in size (Tenebrionidae). The group Chrysomeloidea has this same structure, but has quite highly derived accessory bodies, that differ structurally, each organized in two different regions (crescent-like, and "puff"-like). Among them, moreover, Cerambycidae and Bruchidae have a bilayered acrosomal complex and Chrysomelidae a three-layered complex. In the last families the subfamilies clearly differ for the sperm models, sometimes exhibit classic structure, but sometimes are biaxonemal (*Oulema*). In the subfamily Cassidinae the "puff"-like expansions of accessory bodies fragment into at least five subunits (Donacinae) and sometimes are extremely reduced (Alticinae)¹⁴. Finally, the group Curculionoidea contains species similar to the primitive Chrysomelidae, but also attains in the different subfamilies diverse models for the shape of accessory bodies and acrosomal the complex. Moreover, in Rhynchitinae and Attelabinae the axonemal pattern is "9+9+0"¹⁵. No aflagellate sperm have hitherto been described. Among Oligoneoptera, Hymenoptera (Fig. 6) have a doubtful position. Spermatozoa cannot clarify the problem. Sperm were examined only in a few species of Apocrita, and have a very conventional structure, clearly recalling that of derived coleopterans. They exhibit a bilayered acrosomal complex, 9+9+2 with armed doublets and 16 protofilaments in the wall of accessory tubules, two centrally crystallized mitochondrial derivatives (sometimes of different size), and two, frequently quite small, accessory bodies. More extensive investigation is needed for a definitive statement of the position of the Hymenoptera in the phylogenetic tree of pterygotes.

In conclusion the extremely varied and adaptive evolution of the insect spermatozoon seems to be strictly bound to the phylogenetic tree of the class. Many different systems of motility and egg penetration have been expressed. Each order has its spermatological story that seems to have reached a specific differentiation, giving unexpected and possibly important indications of the origin and evolution of the groups of orders, and of the lower taxa. Some general ideas that this chapter presents are that Protura have evolved independently from unknown ancestors, while Collembola and Japigid Diplurans retain the plesiomorphic characters of the primitive aquatic sperm. From Japigid Diplurans at least two lines of sperm structure are derived: one leads to Campodeid Diplurans, the second directly to the Panorpoid Oligoneopteran complex.

Campodeid Diplurans (or their ancestors) seem to have branched into two lines, one leading to the Thysanuran complex Archeognatha and Zygentoma, the other branching further towards the Palaeoneopteran complex (although extant Ephemeroptera and Odonata are quite different from one other) and towards the Paraneopteran complex, there giving rise to the many hemipteroid orders stopping. After the Thysanuran complex the sperm line seems to have branched further initiating the entire Polyneopteran group and the Neuropteroid complex of Oligoneoptera. Polyneoptera seems to have radiated from the Blattoid stock in many independent lines in which the primary steps seem to be the Gonwandian groups Grylloblattoidea, Cooloolidea, and Eumastacoidea. But Isoptera evolved as a totally separate group, passing through a long series of steps as Protura did among Apterygota. The Neuropteroidea group branched, giving rise to Megaloptera, Raphidioptera, Neuroptera, Strepsiptera, Coleoptera and perhaps Hymenoptera.

But only the sperm of Coleoptera followed long evolutionary path. The panorpoid line arisen directly from japedid ancestors, gave rise to several groups. Initial branches were some Trichoptera, Mecoptera and a few Diptera. From these branches the long lines of Trichoptera, Lepidoptera, Mecoptera, Aphaniptera, and the enormous order Diptera evolved. The view presented herein introduces several new interpretations of insect phylogeny: Polyneoptera, Paraneoptera, panorpoid and neu-

ropteroid complexes seem to have arisen independently from different apterygotan ancestors; Oligoneoptera seems an artificial, polyphyletic group, while Polyneoptera and Paraneoptera seem to maintain their consistency and monophyletism. Moreover as partially presented by Omodeo et al.¹⁶ hemimetabolic and holometabolic insects seem to have similar rank with none being more evolutionary derived than the other.

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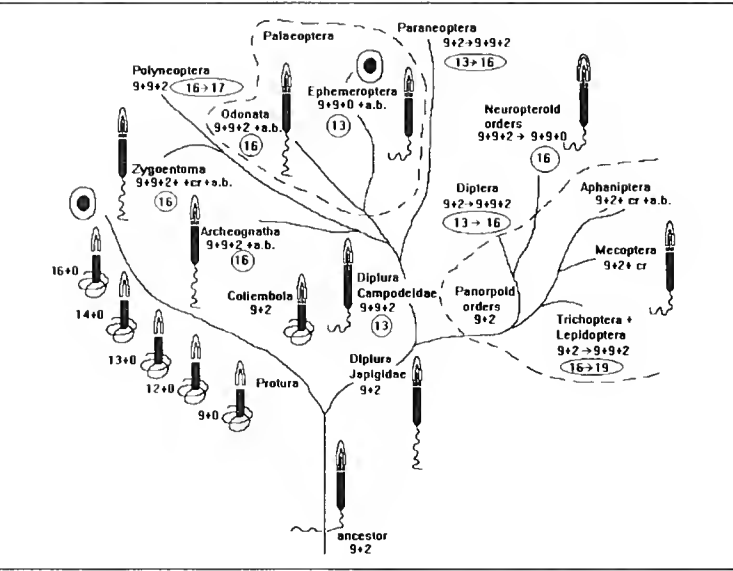


Fig. 1 - A scheme of insect sperm evolution. The symbols represent the elongate, flagellate or, alternatively, the roundish, aflagellate models. The number of layers of the acrosomal complex, as well as the presence of a perforatorium, are designed on the top of the dense nucleus. The axoneme pattern (9+2, 9+9+2, and variations), the presence of crystallomitin in mitochondria (cr), of accessory bodies (a.b.), and, encircled, the number of protofilaments in the accessory tubules (13, 16 a.o.) are also indicated. The further evolution of Polyneoptera, Paraneoptera, Diptera, Trichoptera, Lepidoptera and Neuropteroids is schematized in Figs. 2-6. (From Baccetti, 1).

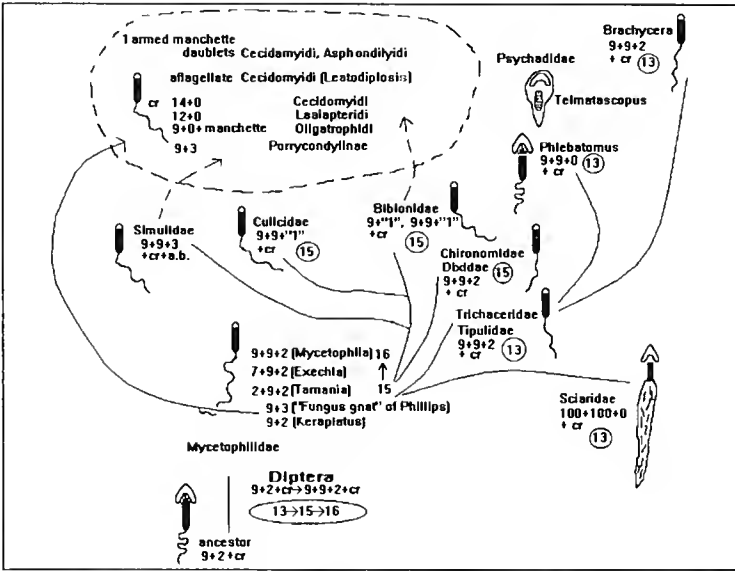


Fig. 4 - Scheme of the evolution of sperm structure in the order Diptera. Observe the enormous variability in axoneme pattern and in the number of protofilaments in the accessory tubules. The mycetophilid *Keroplatus* is the most primitive. From this position *Cecidomyiidae* could have arisen (solid arrow). Other possible origin for this family could be from positions close to *Simuliidae* or to *Bibionidae*. (From Baccetti, 1).

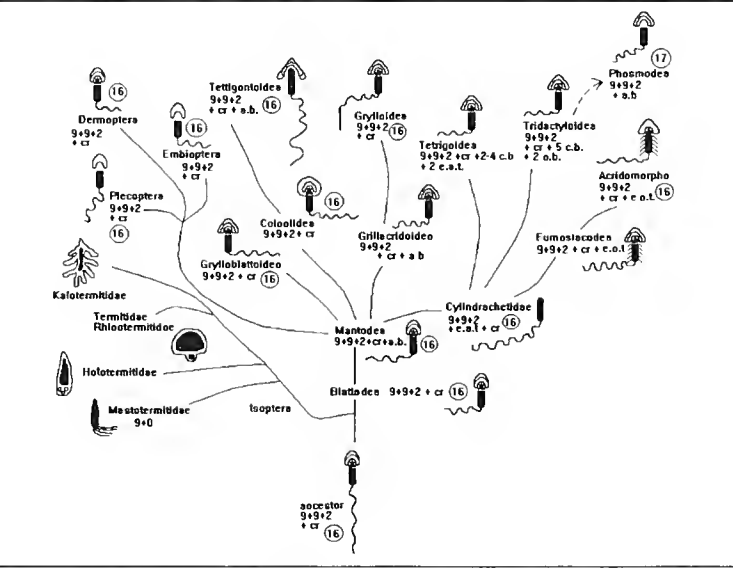


Fig. 2 - Scheme of the sperm evolution in Polyneoptera orders. Symbols are as those in previous schemes. The 9+9+2 pattern, and 16 protofilaments in accessory tubules are generalized. Aflagellarity is limited to the order Isoptera. (From Baccetti, 1).

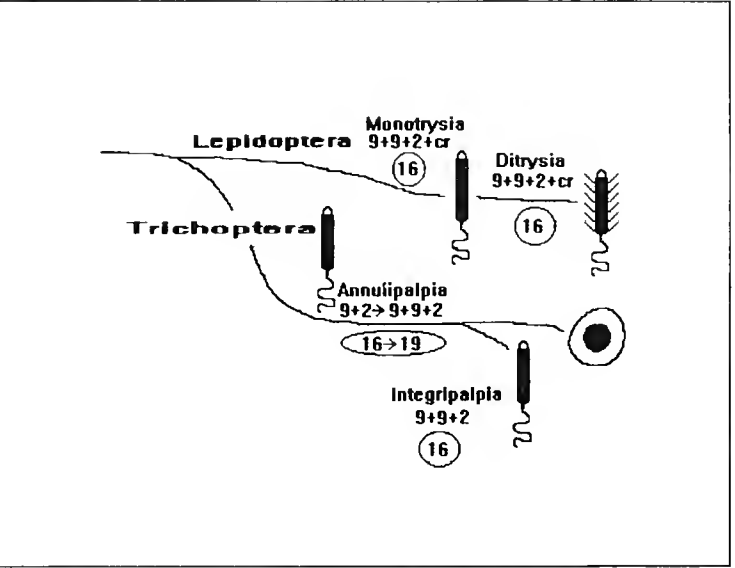


Fig. 5 - Scheme of the sperm evolution in the two groups Trichoptera-Lepidoptera. Symbols are as in previous schemes. The generalized monolayered acrosome, and the lacinate appendages surrounding the ditrysian sperm are represented. (From Baccetti, 1).

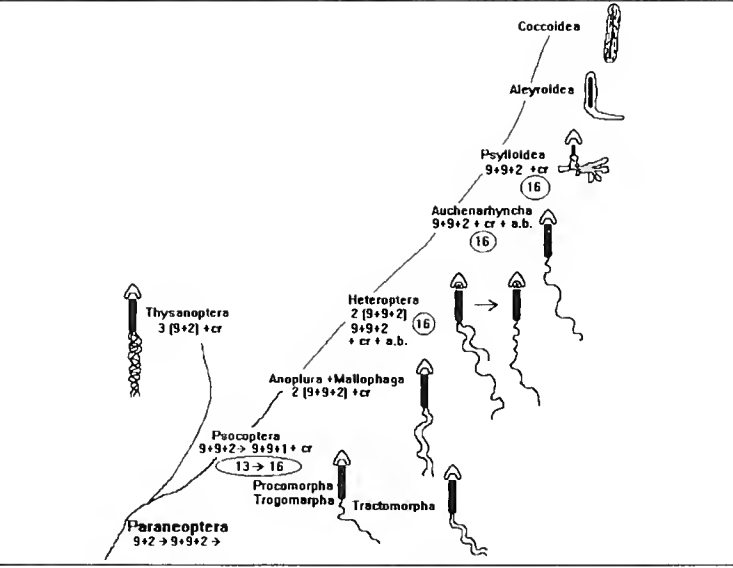


Fig. 3 - Scheme of the sperm evolution in Paraneoptera. The three- or biflagellarity in primitive orders, and the progressive realization of aflagellarity in Homoptera are evident. (Original).

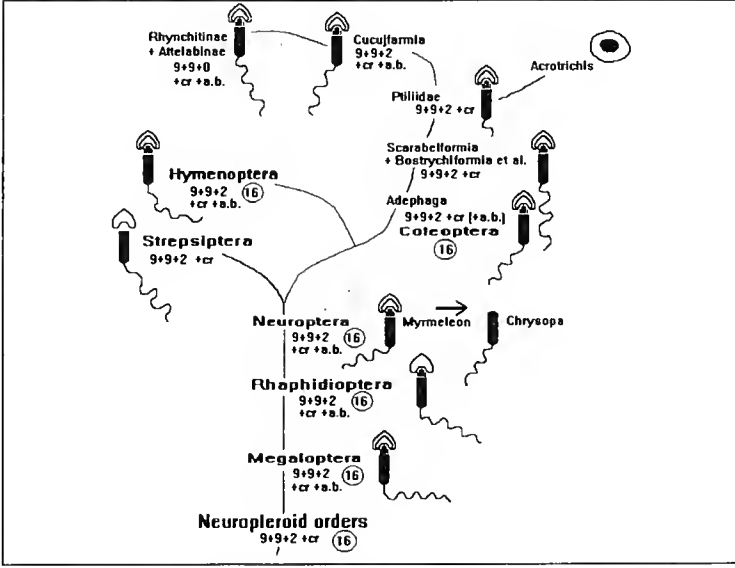


Fig. 6 - Scheme of the sperm evolution in Neuropteroid orders. The group is clearly homogeneous. Only at the top of Coleoptera a few subfamilies having 9+9+0 axonemes, or aflagellate spermatozoa are recorded. (From Baccetti, 1).

MOLECULAR POLYMORPHISMS
AND RATES OF EVOLUTION

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The hypothesis of the molecular clock proposes that rates of molecular evolution are stochastically constant. There is some debate as to how accurate the molecular clock is. It is generally agreed that the variance of evolutionary rates is larger than would be expected by chance, assuming that the probability of nucleotide or amino acid substitution remains constant through time for a given gene or protein. But some authors note that, nevertheless, the fit between the observed and expected number of substitutions is generally good enough, so that the assumption of a clock may be used to make decisions concerning the topology of a phylogeny and the timing of phylogenetic events.

I will, first, present data showing that the evolution of the Cu,Zn superoxide dismutase (SOD) appears to be so erratic that it would lead to erroneous phylogenetic inferences, even after standard corrections are made to account for superimposed and back substitutions. Second, I will extend the SOD analysis to a diverse set of 67 species and show that, if certain restrictive assumptions are made, it is possible to conclude that SOD evolves at a fairly constant rate. An important handicap, however, is that the parametric values of the restrictive assumptions can be estimated only empirically and require large data sets. Hence, phylogenetic inferences based on a limited, or not very diversified number of species may well be in error. Third, I will present data showing that the evolution of glycerol-3-phosphate dehydrogenase (GPDH) is erratic but in a very different manner from SOD. Indeed, in the genus *Drosophila*, the GPDH clock appears to be stuck over an evolutionary span in terms of millions of years (My).

The erratic SOD clock - The superoxide dismutases are essential in the defense of organisms against the toxicity of oxygen. In *Drosophila melanogaster* SOD is a dimer molecule made up of two identical polypeptides, each consisting of 151 amino acids and with a molecular weight of 15,750 daltons. The number of amino acid differences between eight diverse organisms is given in Table 1. These organisms belong to two different kingdoms, fungi and animals, that diverged from each other more than one billion years ago. The fungi include the two remotely related yeast and mold; the animals include an insect, a fish, and four mammals.

Table 1. Amino acid replacements between the SOD sequences of eight organisms

	Rats	Horse	Cow	Fish	Fly	Yeast	Mold
Human	25	30	26	48	58	69	69
Rat		28	23	49	59	63	69
Horse			27	48	61	65	69
Cow				41	60	68	65
Fish					61	67	65
Fly						69	65
Yeast							47

The eight organisms share the same residue at 55 (38.2%) of the 144 sites common to all the sequences. This degree of conservation

is typical of slowly evolving proteins, such as cytochrome *c*. This conjecture of slow evolution is, however, not confirmed when it is noticed that the four mammals, whose ancestral lineages diverged from each other within the last 75 My, share the same amino acid in only 108 (72.5 percent) of their 149 common sites. This is a degree of homology characteristic of very fast evolving proteins.

The phylogenetic history of the eight organisms is shown in Fig. 1. There can be little doubt that the topology of the figure is correct; that is, that the order of branching of the various lineages is as indicated in the figure. The times of divergence shown are less certain. The divergence between the two ungulate lineages or between the rodent and the primate lineages is given as 63 My ago, a figure accepted by paleontologists with a likely error of less than five My. The most recent ancestor to the four mammal lineages is placed at 75 My, a value that could be in error also by a few, but probably no more than 10 My. The divergence between the fish and mammal lineages is placed at 450 My, near the beginning of the Silurian; the ray-fin fishes ancestral to modern fishes and the lobe-fin fishes from which the terrestrial vertebrates derived are already present in the Silurian and become common in the Devonian fossil record. The arthropod and vertebrate lineages had diverged by the beginning of the Cambrian, 570 My. The divergence time of 600 My may be an underestimate, but it is unlikely that it would be by more than 100 My. More tentative yet are the divergence times between the yeast and the mold, given at 600 My on the basis of protein sequence data that assume the molecular clock; and between the two kingdoms, animals and fungi, given as 1,200 My, a figure that could be off by as much as 200 My.

The topology of the phylogeny shown in Fig. 1 makes it possible to group comparisons between pairs of species that are separated by equal lengths of evolutionary time. This has been done in Table 2, where the amino acid differences per hundred residues are given. The corrections are made by the method of Dayhoff (1978, Table 36) but corrections made by other methods, whether for amino acids or for nucleotides do not change in any substantive way the normalized values (Ayala 1986). Cytochrome *c* differences are given for comparison.

It is apparent in Table 2 that there are large discrepancies between the number of amino acid replacements and the times since the divergence of the lineages. This disparity is further exposed by contrasting SOD and cytochrome *c*. The corrected values are fairly similar when we compare the fungi and the animals (58 and 66, respectively); for the comparison between the ungulates, the SOD value is nearly seven times larger than the cytochrome *c* value (19.5 vs. 2.9). Clearly, SOD is not evolving with the kind of regularity seen in cytochrome *c*.

The disparity between the rates of evolution in different lineages and/or different times is further displayed in Fig. 2. When the average differentiation between all four mammals is examined, the rate of amino acid substitution per 100 residues per 100 My is 27.8, a fairly fast rate of evolution. The distances between the swordfish and the mammals or between any of them and *Drosophila* give a rate of evolution of 9.1 amino acid substitutions

per 100 residues per 100 My, which is fairly slow. A very slow rate of evolution is obtained from the distances between the animals and the fungi: 5.5 amino acids per 100 residues per 100 My, similar to the rate obtained when the same comparisons are made for cytochrome *c* (see open circle in the figure), which is a very slowly evolving protein.

Fixing the SOD clock - Fitch and Markowitz (1970) introduced the concept of *covarions* (*concomitantly variable codons*), which asserts that there is a limited number of amino acid sites that can be replaced at any time in a given lineage. The number of sites that can vary remains constant, but the composition of the set varies through time and in different lineages. The application of this assumption to a particular protein requires that we determine (1) the size of the covarion set, i.e., the number of sites at which replacements can occur at any given time in a given lineage; (2) the total number of sites that are variable, i.e., the number of sites, if any, at which amino acid replacements can never occur in any lineage; (3) the number of different amino acids that can occur at the variable sites, which may range from only two different amino acids tolerated at some sites (for example, at a site that must have a negative charge only aspartate and glutamate can occur) to all 20 amino acids; (4) the persistence of the covarion set, i.e., the rate at which one site in the set becomes replaced by another site; and (5) the rate of amino acid replacement. The analysis of 67 SOD sequences from very diverse organisms makes possible to estimate the values corresponding to the five parameters defined in the previous paragraph as follows (Fitch and Ayala 1994): (1) the number of covarions is 28; (2) the number of codons that are permanently invariable across the range of taxa from fungi to mammals is 44; (3) the average number of amino acids that can occur at a variable site is 2.5; (4) the persistence of the covarion set is 0.01; and (5) the rate of amino acid replacement is 0.6 per million years. Table 3 shows that a reasonably good fit is obtained between the observed number of amino acid differences among sequences and the number expected when a molecular clock is operating with the constraints defined by the parameter values just given.

The conclusion derived from the previous analysis is that SOD may be evolving at a constant rate as postulated by the molecular clock, even though it appeared at first quite unreliable because the constraints under which the clock operates had not been taken into account. This conclusion must, however, be tempered by two considerations. The first is that the determination of the components of the clock (such as number of covarions, total set of variable sites, persistence, and number of alternatives allowed per site) requires a very large data set, which may not be available in a particular study. The second consideration challenges the statistical validity of the grounds for accepting the conclusion that a clock is operating, namely the agreement between the observed and expected number of amino acid replacements (Table 3). When the numbers of parameters to be estimated is as large as five and the values that

the parameters can take are not severely constrained, it is small wonder that a reasonable agreement is obtained between the observed values and the expected values so freely estimated.

In any case, a note of caution must be advanced. Inferences of divergence time between lineages derived from a particular clock cannot be assumed to be correct unless the relevant components of the clock have been ascertained. The apparent rate of SOD divergence observed among mammals or flies would yield grossly erroneous time estimates when simply extrapolated to the differences observed between the vertebrate classes (fish versus tetrapod or amphibian versus mammal) or between metazoans and fungi. The SOD “clock” is a complex of several component parts that interact with each other and are subject to various constraints.

Table 3.
Paleontological dates and number of replacements for comparisons between the SOD sequences of 67 diverse species. “Replacements” are the numbers obtained by assuming 0.6 replacements per million years. The expected values are derived from 40 computer simulations for each entry

Comparison	My	Replacements	Amino acid differences	
			Observed	Expected
<i>D. nebulosa</i> - <i>D. melanogaster</i>	55 ± 5	33	8 ± 12	8 ± 12
<i>D. hydei</i> - <i>D. melanogaster</i>	60 ± ?	36	19 ± 3	20 ± 4
<i>Chymomyza</i> - <i>D. melanogaster</i>	65 ± ?	39	23 ± 2	20 ± 4
<i>Homo sapiens</i> - <i>Bos taurus</i>	70 ± 10	42	27 ± 2	22 ± 4
<i>Ceratitis</i> - <i>D. melanogaster</i>	100 ± ?	60	31 ± 2	28 ± 3
Monocot-dicot	125 ± ?	74	28 ± 3	31 ± 5
Angiosperm-gymnosperm	220 ± ?	132	29 ± 7	42 ± 5
Frog-mammals	350 ± 10	210	49 ± 2	53 ± 6
Fish-tetrapods	400 ± 20	240	44 ± 4	56 ± 7
Yeast- <i>Neurospora</i>	?		46 ± 1	
Insect-vertebrate	580 ± 20	348	59 ± 3	60 ± 6
Fungi-metazoans	1,000 ± ?	600	67 ± 4	66 ± 7

The GPDH spastic clock - The enzyme GPDH plays a crucial role in metabolism through its keystone position in the glycerophosphate cycle, which provides energy for flight in the thoracic muscle of *Drosophila* flies. We have obtained in my laboratory the nucleotide sequence of 18 fruitfly species of which 14 species are of the genus *Drosophila*, one species of *Zaprionus* (these 15 species will be referred to collectively as *Drosophila*, since phylogenetically *Zaprionus* falls inside the *Drosophila* cluster), 2 species of the closely related genus *Chymomyza*, and the medfly *Ceratitis capitata*, which belongs to the family *Tephritidae*. Table 4 gives the number of amino acid differences and the nucleotide distances between species, based on 768 coding nucleotides (out of 831 in the complete sequence). The nucleotide distances are estimated using Kimura’s two-parameter method.

There are three triplets of closely related taxa that belong to the same species group: *melanogaster* (1-3), *obscura* (4-6), and *willis-toni* (7-9). The time since the divergence of the species of the same

Table 2.
Number of amino acid differences (per 100 residues) between organisms with increasingly remote common ancestors. Normalized values are shown in parentheses

Comparison	SOD		Cytochrome <i>c</i>		Million years since divergence
	observed	corrected	observed	corrected	
1. Horse to cow and rat to human	17.2 (1.0)	19.5 (1.0)	2.9 (1.0)	2.9 (1.0)	63 (1.0)
2. Horse or cow to rat or human	18.7 (1.1)	20.1 (1.0)	10.6 (3.6)	11.4 (3.9)	75 (1.2)
3. Fish to mammal	31.0 (1.8)	40.0 (2.1)	18.3 (6.3)	20.9 (7.2)	450 (7.1)
4. Insect to vertebrate	39.9 (2.3)	55.9 (2.9)	20.0 (6.9)	23.3 (8.1)	600 (9.5)
5. Yeast to mold	30.7 (1.8)	39.4 (2.0)	37.5 (13.0)	52.5 (18.2)	600 (9.5)
6. Fungus to animal	44.6 (2.6)	66.2 (3.4)	40.9 (14.2)	58.0 (20.0)	1,200 (19.0)

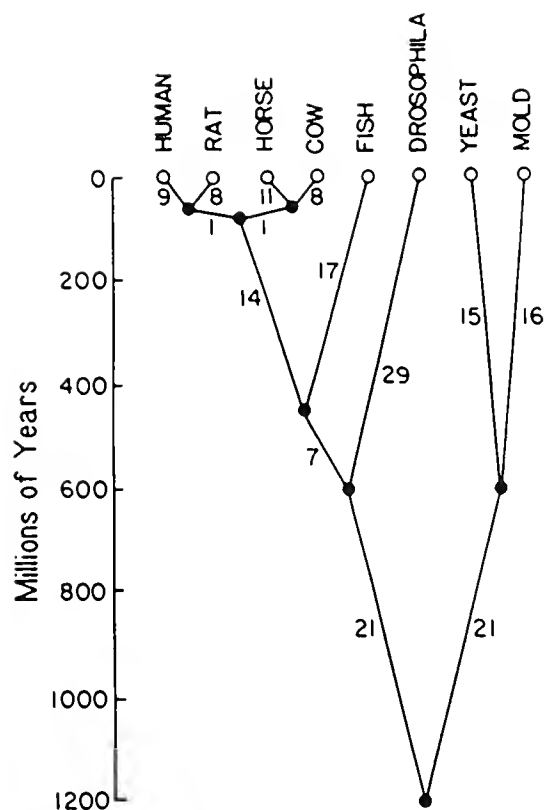


Figure 1. Phylogenetic relationships among eight organisms with the approximate time scale. The numbers along the branches of the phylogeny represent the inferred numbers of amino acid substitutions that have taken place per 100 residues in the evolution of SOD.

group is below 20 My; there are no amino acid differences among species of the same group. The time of divergence for all other *Drosophila* comparisons ranges from 40 to 65 My; the average number of amino acid differences is about 3, with little correlation between the divergence time and the number of differences, as it is apparent in Fig. 3 (left). The divergence between *Chymomyza* and the *Drosophila* species is estimated at 65 My; it occurred just about the same time as *D. lebanonensis* diverged from the other *Drosophila* species. Yet the number of amino acid differences between *Chymomyza* and *Drosophila* is about 8, more than twice the average number of differences among the *Drosophila* species. The divergence between *Ceratitidis* and the *Drosophilidae* occurred about 100 My; the number of amino acid differences is about 21, seven times larger than among the *Drosophila*.

The spastic character of GPDH evolution is shown in Fig. 3 (left). The rate of amino acid replacement per hundred million years is 2.1 for comparisons between *Drosophila* species, 4.4 between *Drosophila* and *Chymomyza*, and 10.3 between *Ceratitidis* and the *Drosophilidae*. The long-term rate of evolution of GPDH based on comparisons between species from three animal phyla (insects, mammals, and nematodes) or from three kingdoms (animals, plants, and fungi) is approximately the same as between *Ceratitidis* and *Drosophila*, about 10 replacements per hundred million years. The pattern of GPDH amino acid evolution over time is,

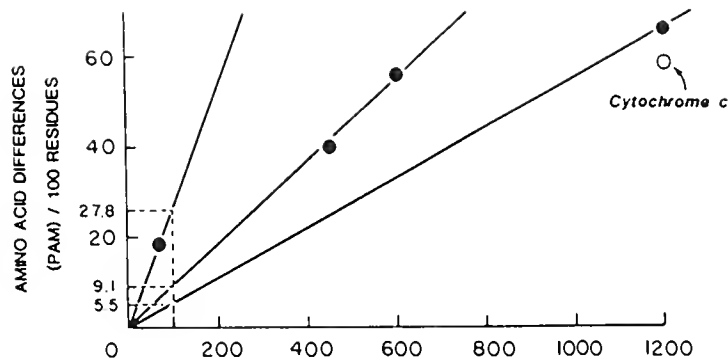


Figure 2. Rate of amino acid substitutions per 100 residues in the evolution of SOD. From left to right, the solid circles represent, respectively, the average differentiation between four mammals (70 My), between the swordfish and the mammals (450 My), between the fruit fly and the five vertebrates (600 My), and between two fungi and the six animals (1,200 My). The three straight lines give three different rates of evolution: 5.5, 9.1 and 27.8 amino acid substitutions per 100 residues per 100 My. The open circle represents the average differentiation between the two fungi and the animals for cytochrome c, a protein known to evolve at a fairly constant rate.

thus, the reverse of the pattern observed in SOD. In GPDH, the apparent long-term rate is about five times faster than the short-term rate; in SOD it is five times slower. It may be noticed that, nevertheless, the rate of nucleotide substitutions in *Gpdh* conforms fairly well to the expectations of the molecular clock (see Table 4, below the diagonal, and Fig. 3, right).

It may well be possible to fit the rate of GPDH evolution to the hypothesis of the molecular clock by making an appropriate set of assumptions and estimating parameter values derived from the observations at hand. But will these assumptions and parameter values obtain when other taxa are investigated? The answer is “no,” at least in the case of GPDH. The rate of amino acid replacement between mammal species that diverged up to 65 My ago is much greater than between *Drosophila* species; rather, it approximates the long-term rate of GPDH evolution.

In conclusion, I will reiterate the point made earlier. The molecular clock is a powerful hypothesis when applied to the reconstruction of phylogeny. It may often be the most reliable among available sources of evidence, but it may also lead to very erroneous inferences. The molecular clock, to the extent that there is one, is a complex clock with various components and constraints that cannot be specified a priori, nor can they be extended from one to another group of organisms, much less from one to another gene.

Table 4.
Number of amino acid differences (above the diagonal) and nucleotide distances (x1,000, below the diagonal) between Gpdh

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. <i>D. melanogaster</i>	-	0	0	2	2	2	2	2	2	3	2	2	0	4	3	9	8	21
2. <i>D. simulans</i>	12	-	0	2	2	2	2	2	2	3	2	2	0	4	3	9	8	21
3. <i>D. teissieri</i>	38	27	-	2	2	2	2	2	2	3	2	2	0	4	3	9	8	21
4. <i>D. pseudoobscura</i>	165	166	180	-	0	0	2	2	2	3	2	4	2	4	3	9	8	22
5. <i>D. miranda</i>	163	164	177	4	-	0	2	2	2	3	2	4	2	4	3	9	8	22
6. <i>D. guanche</i>	162	166	185	48	53	-	2	2	2	3	2	4	2	4	3	9	8	22
7. <i>D. willistoni</i>	168	166	180	152	153	157	-	0	0	3	2	4	2	5	5	9	8	21
8. <i>D. paulistorum</i>	166	164	178	155	157	159	15	-	0	3	2	4	2	5	5	9	8	21
9. <i>D. nebulosa</i>	185	187	192	168	170	159	60	65	-	3	2	4	2	5	5	9	8	21
10. <i>D. virilis</i>	179	171	189	152	153	146	146	137	162	-	1	5	3	4	4	8	7	21
11. <i>D. hydei</i>	175	168	182	147	145	154	160	158	171	99	-	4	2	3	3	7	6	20
12. <i>D. busckii</i>	164	155	173	170	171	164	137	134	148	119	132	-	2	5	3	8	7	20
13. <i>D. pictiventris</i>	197	191	195	166	168	179	143	145	168	141	141	146	-	4	3	9	8	21
14. <i>D. lebanonensis</i>	203	200	211	182	188	182	164	163	178	181	174	174	185	-	5	9	7	21
15. <i>Z. tuberculatus</i>	167	164	175	158	159	158	150	152	167	113	118	138	143	184	-	6	5	20
16. <i>C. amoena</i>	256	257	161	212	214	207	205	203	203	193	222	199	189	192	206	-	2	20
17. <i>C. procnemus</i>	243	244	252	208	210	210	195	194	197	207	217	190	197	193	206	105	-	19
18. <i>Ceratitis</i>	274	272	296	287	284	283	255	263	274	271	274	158	285	269	265	310	268	-

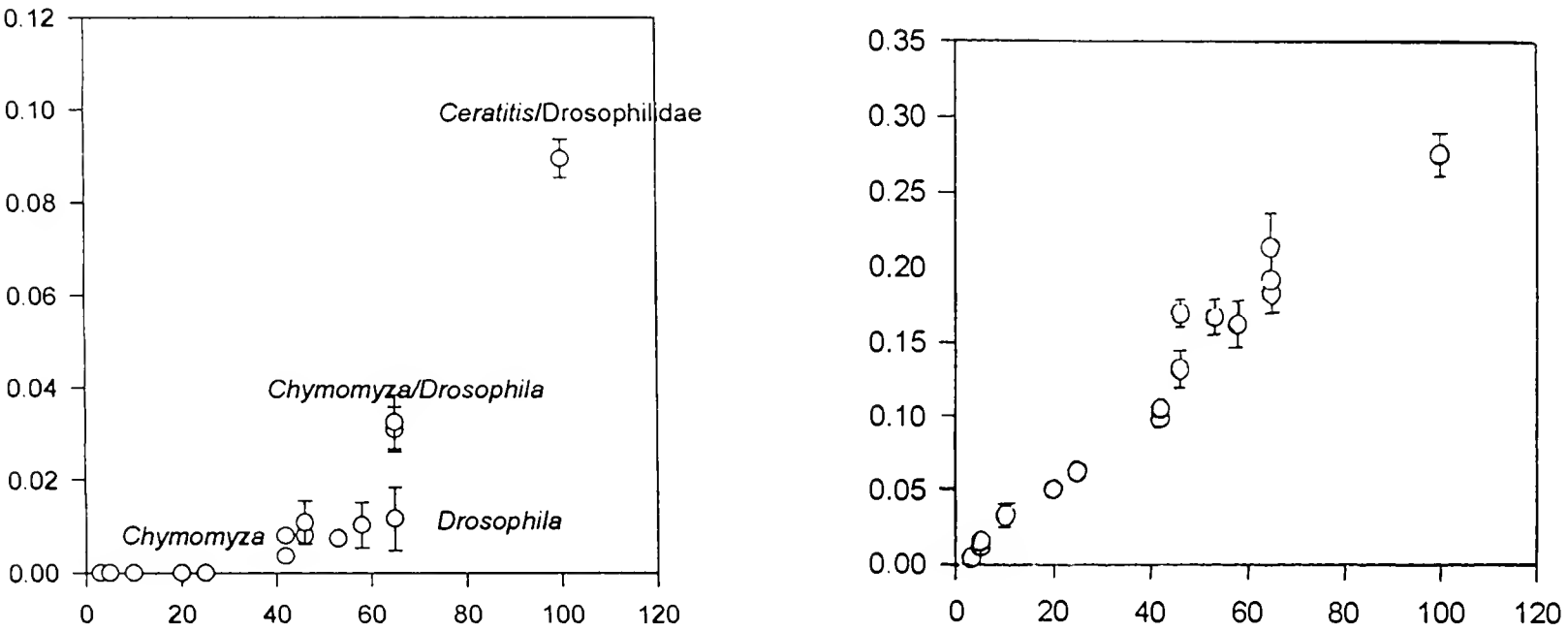


Figure 3. Rate of amino acid (left) and nucleotide (right) evolution in fruitflies. The scale at bottom is in million years. Amino acid distances are estimated by the method of Dayhoff (1978); nucleotide distances are estimated by the method of Kimura (1980).

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INSECT EVOLUTIONARY BIOLOGY: LESSONS FROM DROSOPHILA

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Drosophila have long served as a model organism for a wide variety of biological research especially in genetics and evolution. The popularity of this model has reached its highest peak in recent years with more scientists working on more different aspects of its biology than ever before. The fields of *Drosophila* development, neurobiology/behavior, and molecular biology have seen the greatest growth in the last fifteen years. The numbers of publications on *Drosophila* has been rising exponentially for the last 70 years with well over 70,000 published papers today. This growing knowledge will be particularly important in evolutionary studies as any full understanding of evolution must involve knowledge at all levels of biological organization. Reciprocally, evolutionary understanding can shed light on all the other fields because evolution is the organizing umbrella under which all biological information can be best understood. The purpose of this lecture is to review the present status of evolutionary studies on *Drosophila* and to emphasize generalities which are very likely to hold true for a large part of the class Insecta. A fuller account can be found in a forthcoming book titled *Progress and Prospects in Evolutionary Biology: The Drosophila Model* (to be published by Oxford University Press).

The genus Drosophila - When most biologists think of "Drosophila" they think of *D. melanogaster*, the most widely studied species. However, there are about 2,000 species in the genus that occupy virtually every region of the world with the exception of there being no truly Arctic *Drosophila*. The genus very likely originated in Southeast Asia some 60-70 mya (million years ago). There are three major subgenera of the genus: *Sophiophora* (about 300 species including *D. melanogaster*), *Drosophila* (about 700 species), and *Idiomyia* (800-1,200 species). The last is a monophyletic group of endemic Hawaiian *Drosophila* that has irradiated to form the greatest morphological and behavioral diversity of all the *Drosophila*. Interestingly, it split from subgenus *Drosophila* about 30 mya, thus predating existing islands (Powell and DeSalle, 1995). Most likely the colonization occurred on islands of the archipelago that are now submerged (Beverley and Wilson, 1985).

The larval breeding sites of *Drosophila* species are generally rotting, fermenting vegetation including fruits, stems, fluxes, leaves, bark, cacti, and fungi. Yeast and bacteria growing on these substrates are a primary source of nutrition. There also exist some bizarre species which breed in the spittle of spittle bugs (Cercopidae), nests of solitary bees, and parasitize spider eggs. Three independent times, a species has adapted to breeding in the nephritic groove of crabs (Carson, 1974). Perhaps the most unusual are seven species in Africa which predate aquatic larvae of blackflies and midges. Unfortunately, many of the most used species in laboratory research are those that have become adapted to human refuse and thus have rather boring ecologies. It needs to be emphasized that the species most studied in laborato-

ries are a small subset of species selected for those species easily available and able to breed on artificial food; these have most often been "garbage species" such as *D. melanogaster*.

Visible mutations - Early in the history of genetics, it was not clear whether the morphological mutants cropping up in laboratory cultures and being genetically studied were simply laboratory artifacts or actually exist in natural populations. A large number of studies have examined frequencies of visible mutations in natural populations and the general conclusion is that many of the laboratory-identified mutations can be found in nature, but their frequency is very low (Spencer, 1947). Approximately one visible mutation occurs per two haploid genomes in nature. The number of loci capable of producing a discretely visible phenotype is estimated to be about 2,000, so the average frequency in a natural population of any single visible mutation is about 0.00025. The message is that overwhelmingly, visible mutations are deleterious and never reach appreciable frequencies in populations. In light of recent molecular findings, this is not unexpected: most visible mutations are caused by insertion of a transposable element that destroys or radically alters gene function.

Molecular variation - Underlying this remarkable morphological homogeneity in *Drosophila* populations is a plethora of genetic variation. This was first demonstrated using allozymes when it was determined that about 50% of all loci studied are polymorphic for alleles in high frequencies and any individual is heterozygous at about 15% of all loci (Powell, 1975). It was clearly recognized that this must be a serious underestimate as only nucleotide changes that cause a change in electrophoretic mobility of proteins are detected. More recent DNA sequencing has confirmed this serious underestimate. Estimates of per nucleotide heterozygosity range from 0.5 to 1% for coding DNA, and 1 to 2% for non-coding DNA (Moriyama and Powell, 1996). In *Drosophila* 98% of the genes are over 200 bp with a mean of about 1,700 bp (coding region only). This means, that in addition to every individual being genetically unique, *each is heterozygous at virtually all loci!* Of course, it remain to be shown how much of this nucleotide variation has adaptive meaning, although the growing evidence is that much of it is subject to selective forces, even synonymous mutations that do not affect proteins.

Inversion variation - A special type of naturally occurring variation that is especially ubiquitous and important in *Drosophila* are chromosomal inversions, especially paracentric inversions (Krimbas and Powell, 1992). Of the species examined, 58% (106/183) are polymorphic for naturally occurring inversions. The knowledge of inversions in *Drosophila* is greatly aided by the giant polytene chromosomes. I suspect many other insects would be equally variable for these variants if they were examined and

favorable cytogenetic material were available.

Two aspects of inversions are especially important: (1) they suppress recombination within the breakpoints and (2) they are often under extremely strong selection. If one is working with a species and is not aware of the presence of inversions, considerable misinterpretation of data might ensue because of these two properties. For example sequencing a gene for systematic purposes could be very misleading if unbeknownst to the researcher, that gene is within an inversion (e.g., Powell, 1991).

Speciation - Considerable progress has been made in understanding the genetic basis of speciation in *Drosophila* (Coyne, 1992). Several points are especially worth noting. First, multiple examples exist of cases of either pre-mating or post-mating isolation occurring first in the speciation process; there seems to be no general rule. Second, Haldane's rule holds very well in *Drosophila*: in interspecific hybrids, if only one sex is sterile or inviable, it is the heterogametic sex (males). Third, when post-mating isolation is the first step in speciation, male sterility is the most frequent form. Fourth, the genetic cause of sterility is most often highly multigenic while inviability may have a less complex cause, i.e., fewer genes are involved (Wu and Davis, 1993). The X chromosome has a disproportionate effect for both sterility and inviability likely due to recessive mutations being expressed in the hemizygous heterogametic sex. Fifth, exceptions to highly multigenic causes of reproductive isolation may occur for behavioral isolation where single genes may have a major effect on courtship song or pheromone production. Sixth, founder effects are one documented population process that can start the speciation process; most often the first isolation induced by founder effects is premating isolation (Powell, 1989). Following a founder event, often the mating discrimination is asymmetric with derived females displaying less discrimination against ancestral males than do ancestral females discriminate against derived males (Kaneshiro, 1976).

Phylogenetics - Given the large number of species in a single genus, the small size, and relative lack of morphological variation, it is not surprising that understanding of the phylogenetic relationships among species and species groups is very incomplete. However, considerable progress has been made recently by using DNA data to construct molecular phylogenies (Powell and DeSalle, 1995). Such phylogenies can be helpful in understanding evolutionary principles. As just three examples in *Drosophila*, we can demonstrate (1) that chromosomal arm fusions are much more common than fissions, (2) intron loss is most often a derived character for a gene, and (3) transposable elements can be transferred across species boundaries.

Genome evolution - Genome size in *Drosophila* varies by about a factor of six. By far the majority of change occurs for heterochromatin. There is no correlation between body size and genome size. Chromosomal arm content has remained remarkably constant through the evolution of the species with the same genes remaining on the same arm, although arms are rearranged by paracentric inversions. The evolution of sex chromosomes can be studied in *Drosophila* as there are cases where former autosomes have become attached to a Y chromosome. Such autosomes begin to become genetically inactive due largely to the accumulation of transposable elements (Steinemann et al, 1993). The homologue of the attached autosome becomes a neo-X because it is hemizygous in males; it evolves dosage compensation.

Molecular evolution - As previously mentioned, *Drosophila* have been well-studied for naturally occurring molecular variation, with considerable being found. An exception to high levels of nucleotide variation is for regions of the genome with very low recombination, e.g., the non-recombining dot fourth chromosome of *D. melanogaster* has no documented variation (Berry et al, 1991). This is likely due to hitchhiking effects caused by either selective sweeps of advantageous variants or selection against chromosomes with deleterious mutations. X-linked genes are less variable than autosomal genes, likely because the population size of X chromosomes is 3/4 that of autosomes. Polymorphisms involving transitions outnumber those for transversions, likely because of the structure of the genetic code: transitions are more often synonymous substitutions than are transversions. Codon usage bias is often extreme in *Drosophila* and specific to certain codons. A single gene (e.g., *Alcohol dehydrogenase*) may display phylogenetic persistence of avoidance of codons throughout the genus. It is impossible to explain the pattern of codon usage bias in *Drosophila* without invoking a role for natural selection detecting these mutations.

In comparing patterns of variation within and between species, differences are seen in the level of synonymous and replacement changes. The ratio of these is higher within than between species (McDonald and Kreitman, 1991). A possible explanation is that replacement mutations are either deleterious and quickly eliminated; or they are advantageous and quickly swept to fixation. In either case they are unlikely to be observed in a polymorphic state. Synonymous mutations are closer to neutral and thus can remain polymorphic in a population for longer periods and thus likely to be observed as polymorphisms.

Development - While considerable progress has been made in understanding the molecular and genetic control of early development in *Drosophila*, little has been learned about the evolution of development. One problem has been that the majority of work has been done on early development when the overall *Bauplan* is laid down. From species comparisons, this appears to be invariant throughout the genus. To find significant variation it has been necessary to go outside Diptera, which makes evolutionary comparisons very difficult. To understand the evolutionary history of traits one needs closely related monophyletic lineages such as exist within *Drosophila*, where no variation has been found. It is likely that the majority of diversity in *Drosophila* is caused by later stages of development which have not been well-characterized. It is also possible that little or no variation in the early stages of development is due to the fact that all the molecular probes and theoretical orientation have been derived from a single species, *D. melanogaster*. If other species were using different developmental programs, would we recognize it?

Phyletic phenocopies - More interesting from an evolutionary standpoint has been the suggestion that some of the diversity of *Drosophila* is due to rather simple changes in major genes. The concept of phyletic phenocopies states that different lineages may differ by traits known to be mutants in one species. This not only helps in understanding the possible genetic basis of morphological diversity, but also may help in systematic studies. Examples include Hawaiian *Drosophila* (DeSalle and Carew, 1992): the broad head of *D. heteroneura* is similar to *Deformed* and *labial* mutants of *D. melanogaster*; the modified mouth parts group resemble *proboscipedia* mutations; the modified tarsi group have appendages similar to *Distal-less* mutations. The absence of interfacetal setae was once used as evidence that Hawaiian *Drosophila* do not belong in the genus *Drosophila*, but

once it was recognized that mutations in the single gene *Hairless* can give rise to that trait, its importance as a phylogenetic character was downgraded.

This very brief survey of recent, and some not so recent, advances in knowledge of *Drosophila* evolutionary biology should give some indication of the richness of the studies on this

single genus. Many of these findings have general applicability to all animals, more to all insects, and even more to all Diptera. I urge you to look to the *Drosophila* literature for guidance as to how to approach your own particular problem and what you might expect to find. *Drosophila*, too, is an insect.

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LIFE HISTORY EVOLUTION IN INSECTS: THE ROLE OF THE PHENOTYPE IN EVOLUTIONARY THEORY

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Great theories make surprising predictions. By adding phenotypes to evolutionary theory, life history evolution extended the range of predictions from hard-to-measure genetic patterns to easier-to-measure whole-organism traits directly connected to fitness. It has successfully explained: why organisms are small or large, why they mature early or late, why they have few or many offspring, why they have a short or a long life, and why they must grow old and die.

This talk has four parts. I will first review how life history theory explains the evolution of (1) age and size at maturity, (2) reproductive investment, and (3) lifespan and ageing, then (4) give you some evidence and concluding thoughts on two concepts in life history evolution that are not yet fully understood: tradeoffs and phylogenetic constraints. You will see that theoretical biology has produced surprising insights confirmed by successful quantitative predictions, that life history evolution plays a central role in evolutionary theory, that tradeoffs and constraints remain a problem, and that evolution is becoming a great scientific theory with the same status as physics.

(1) *Age and size at maturity* - Age and size at maturity are optimized when the benefits and costs of maturation at different ages and sizes balance at a stable equilibrium point or along a reaction norm. We start with an abstract picture of a life history, define the costs and benefits of delaying maturity, model optimum age and size in one environment, then extend the model to reaction norms for age and size in changing environments. This example makes three important points: (a) the quantitative predictions were successful, (b) the modeling procedure was simple, and (c) traits involved in tradeoffs contribute most to fitness at intermediate values.

(2) *Optimal clutch size and reproductive investment* - David Lack pointed out that if nestling survival decreases linearly as clutch size increases, then an intermediate number of eggs produces the most fledglings. He was right in theory, wrong on the details, but he got the main point: fitness is often maximized at intermediate reproductive investments. Some of the best recent work on clutch size has been done on kestrels in The Netherlands. If kestrels lay the optimal clutch, then larger or smaller clutches should have lower fitness than the one actually laid. When Serge Daan, Jos Tinbergen, and Cor Dijkstra manipulated clutch size in kestrels, they found that kestrels optimize their reproductive investment with clutches of intermediate size. This confirmed Lack's view, and their work also showed that if you want to measure reproductive costs, you have to be prepared to follow individuals for at least two generations, for many of the costs are paid by the offspring.

The problem of clutch size can be generalized to the problem of predicting the optimal allocation to reproduction over the entire lifespan. This is referred to, in life history theory, as The

General Life History Problem or as The Reproductive Effort Model. Some important predictions of the Reproductive Effort Model: (a) if mortality rates increase in one adult age class, then the optimal reproductive effort increases before that age and decreases after it (Michod 1979); (b) if adult mortality rates increase, the optimal age at maturity decreases (Roff 1981); and (c) if mortality rates increase in all age classes, then optimal reproductive effort increases early in life and optimal age at maturity decreases (Charlesworth 1980). Now let us consider two tests of these predictions:

the first test was done by David Reznick, John Endler, and their colleagues, who changed guppy mortality rates for 13 years by manipulating predators in the field in Trinidad. The guppies responded to increased mortality by maturing earlier at a smaller size and producing more, smaller offspring when raised under constant conditions through two generations in the laboratory (the changes were genetically based). Evolution happened very fast - these differences were significant within 50 generations.

A second test is currently being carried out in my laboratory. It involves a selection experiment on *Drosophila*, and the part of that experiment that tests The Reproductive Effort Model consists of two treatments each with three replicates. The first treatment is high adult mortality - 90% of the flies in the cage are killed and replaced twice per week; the probability of surviving one week as an adult is 0.01. Juvenile mortality is about 96.6%. The second treatment is low adult mortality - 20% of the flies in the cage are killed and replaced twice per week; the probability of surviving one week as an adult is 0.64. Juvenile mortality is about 99.2%. Under the high adult mortality treatment, the main problem faced by the flies is to achieve peak fecundity at 14 days of age when they enter the cage with only 24 hours to live. At 25° they normally achieve peak fecundity at 15-16 days. Thus they need to move that peak forward 1-2 days, and one way to do so is to accelerate development and eclose earlier at a smaller size.

Results of the selection so far: (1) females from the high adult mortality treatment develop 9-12 hours faster than females from the low adult mortality treatment, are about 3% lighter, have higher fecundity early in life and lower fecundity later in life. Males from the high adult mortality treatment develop 14 hours faster but do not weigh less than males from the low adult mortality treatment, suggesting that the age-size tradeoff itself has evolved to compensate. This is precisely what was predicted by The Reproductive Effort Model.

(3) *Reproductive lifespan and ageing* - Reproductive lifespan is a balance between selection to increase the number of reproductive events per lifetime and effects that increase the intrinsic sources of mortality with age. The first lengthen life, the second shorten it. Part of the increase in intrinsic mortality rates with age is due to tradeoffs common to all members of a species

(physiology caused by phylogenetic constraints) and part is due to the genetic effects that result in ageing or senescence. The process that produces an optimal lifespan given phylogenetically fixed tradeoffs works like this: selection pressures that lengthen life decrease the value of offspring and increase the value of adults. These include lower adult mortality rates, higher juvenile mortality rates, and increased variation in juvenile mortality rates from one reproductive event to the next.

In addition to the optimisation of the lifespan through life history evolution, we must also take into account the evolution of ageing. Organisms that reproduce sexually, have a clear separation of germ line from soma, and return to a single-cell stage at some point in their life cycle must age. Organisms that reproduce by simple division should not age. The evolutionary theory of ageing starts by observing that selection pressures drop with age; sufficiently old organisms are irrelevant to evolution. This fact has two consequences: first, genes with positive effects early in life and negative effects late in life are selected for and accumulate in the genome. Their effects are called antagonistically pleiotropic. Second, more deleterious mutations should accumulate and be maintained by selection-mutation balance when they are only expressed in older, rather than younger, age classes, for in older ages the force of selection is smaller and allows the accumulation of more mutations in a classical mutation-selection balance. Thus ageing is seen by evolutionary theory as a byproduct of selection for reproductive performance. It arises through the accumulation of many genes that have positive or neutral effects on fitness components early in life and negative effects on fitness components late in life.

Drosophila is one of the most important model systems for the evolution of ageing. Experiments on *Drosophila* suggest that early and late fitness components do trade off, but whether lifespan trades off with early fecundity or with age and size at maturity depends on the treatment and the lab doing the work.

(4) *Some observations about tradeoffs and phylogenetic constraints* - We do not yet have a single case in which the major

causes that determine tradeoffs have been separated experimentally to estimate their relative importance. Those causes include phylogenetic heritage, primarily expressed as development and physiology common to all individuals in a species, and genetic variation among individuals. We have performed a genetic engineering experiment in which we appear to have revealed the influence of phylogenetic constraints while investigating the impact of genetic variation. Those manipulations revealed the same sorts of tradeoffs that one finds in selection experiments. This raises an important question: is the expression of genetic variation influenced by the “fixed” part of the genome, by physiology and development? If it is, that would explain the origin and impact of phylogenetic constraints.

Life histories are probably not the best system in which to ask this question at the moment. A better one is the patterning of butterfly wings, a system currently being developed by Paul Brakefield, Vern French, and Sean Carroll.

Some points to remember - (1) Traits were analyzed one-by-one: age and size at maturity, reproductive investment, lifespan and ageing. (2) The analysis was done in terms of mortality rates, fecundity rates, and tradeoffs. (3) Reliable studies have shown important tradeoffs among age and size at maturity, reproductive investment, and lifespan. (4) These tradeoffs are the basis for coordinated syndromes of life history traits - not habitat templates, not r&K selection.

Conclusion - Life history traits determine fitness. Individual variation in life history traits is natural selection. Life history evolution has a central place in evolutionary theory because it makes clear the mechanism of natural selection and the potential reactions to it. It has put phenotypes back in the center of evolutionary theory. Theoretical biology has produced surprising insights confirmed by quantitative tests.

Life history theory is helping evolution to become a scientific theory with the same general intellectual status as the great theories of physics.

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NEUROETHOLOGY OF SEX- AND HOST-ATTRACTION IN MOTHS

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In moths and other insects, olfaction plays a major role in the control of important behaviors. Orientation and movement toward, and interactions with, receptive mating partners, appropriate sites for oviposition, sources of food, and hosts for parasitism typically involve olfactory signals that initiate, sustain, and guide the behaviors. Because of their prominence in the zoosphere, their economic and medical importance, and their usefulness as models for both behavioral and neurobiological research, insects have been extensively studied by investigators interested in mechanisms of olfactory control of behavior.

Insects respond to a variety of semiochemicals, including pheromones (chemical messengers within a species, such as sex attractants) and kairomones (chemical messengers between species and adaptively favorable to the recipient, such as attractants and stimulants for oviposition and feeding emitted by a host plant). Studies of insect responses to such biologically significant odors have shown that the quality and quantity of odorants in complex mixtures present in the environment are encoded in patterns of activity in multiple olfactory receptor cells (ORCs) in the antennae. These "messages" are decoded and integrated in the olfactory centers of the central nervous system (CNS), and it is there that olfactorily induced changes in the behavior or physiology of the insect are initiated.

Of paramount interest, both historically and currently, is the attraction of a mating partner by means of a chemical signal - the sex pheromone - released by a receptive individual of one sex and detected by conspecifics of the opposite sex. In moths, these chemical signals are the primary means by which females broadcast their sexual receptiveness over relatively long distances to conspecific males. The male moths respond to the sex-pheromonal stimulus with well-characterized mate-seeking behaviors involving patterned anemotactic flight, short-range orientation to the calling female, and mating.

Behaviorally relevant olfactory mechanisms have been probed in depth in several insect taxa, including cockroaches, honey bees, and fruit flies, but the most extensive explorations of the detection and processing of, and behavioral responses to, sex pheromones have been carried out in moths. Building upon earlier and recent work of others (e.g. References from 1 to 7) and paralleling the current research in many laboratories using different insect species, my coworkers and I study the olfactory system of the experimentally favorable giant sphinx moth *Manduca sexta*. We have investigated this model olfactory system extensively by means of anatomical, neurophysiological, biochemical, cytochemical, developmental, and cell-culture methods. This brief overview focuses on some of our findings and speculations about the functional organization and physiology of the olfactory system in *M. sexta*. More detailed reviews of this and other aspects of our work have been presented elsewhere⁸⁻¹², and our colleagues have made significant progress in studies of the pre-motor and motor aspects of the neuroethology of odor-guided

behavior as well as the behavior itself¹³⁻¹⁷.

A principal long-term goal of our research is to understand the neurobiological mechanisms through which information about a specific olfactory signals, the female's sex pheromone and the volatiles released by host-plants, are detected and integrated with inputs of other modalities in the brains of male and female moths, respectively, and how these messages ultimately initiate and control the characteristic behavioral responses of the moths. Pursuit of that goal promises to teach us much about how the brain processes chemosensory information and uses it to shape behavior. Our studies along this line to date have persuaded us that the male's olfactory system consists of two parallel sub-systems, one a sexually dimorphic "labeled-line" pathway specialized to detect and process information about the sex pheromone, and the other a more complex pathway that processes information about plant (and probably other environmental) odors encoded in "across-fiber" patterns of physiological activity. In this presentation our work on the male's olfactory system will be emphasized, but current studies of female moths will also be touched upon. Major features of the organization of the male moth's olfactory pathways are schematized in Fig. 1.

Olfactory primary afferents - Each of the 2 antennae of adult *Manduca* comprises 2 basal segments, which contain mechanosensory organs, and a long sexually dimorphic flagellum divided into ≥ 80 annuli (segment-like divisions) bearing numerous sensilla of several types, the great majority of which are olfactory. Antennal flagella of adult males and females have many ORCs (associated mainly with basiconic, coeloconic, and trichoid sensilla) that respond to plant volatiles.

Sexually dimorphic sensilla on male antennal flagella have been partly characterized in *Manduca*. Each male antenna has a total of $>3 \times 10^5$ ORCs associated with ca. 10^5 sensilla, of which ca. 40% are male-specific type-I trichoid (hair-like) sensilla^{18,21}. Type-I trichoid sensilla (100-600 μm long) are typical olfactory sensilla²², each innervated by 2 ORCs whose dendrites extend through the lumen of the fluid-filled hair to its tip. In most of these sensilla, one of the 2 male-specific ORCs is highly sensitive and specific to one of the 2 necessary and sufficient components (E10,Z12-hexadecadienal or component A) of the female's sex pheromone, while the second ORC is "tuned" to the second essential component (E10,E12,Z14-hexadecatrienal or component B)^{19,23,24}. These pheromone-specific ORCs thus have extremely narrow molecular receptive ranges, and each type collectively represents a narrowly tuned input channel that conveys information about the presence, concentration, and temporal patterning of one key pheromone component to the brain.

Antennal lobe and glomeruli in the adult - Ordinary glomeruli. The axons of antennal ORCs project, via the antennal nerve, strictly ipsilaterally and define 64 ± 1 large (50-100 μm in

MGC to higher olfactory centers³²⁻³⁹. PNs that respond preferentially to stimulation of the antenna with component A have arborizations restricted to the toroid, whereas PNs that respond selectively to stimulation with component B have arborizations confined to the cumulus³⁹. PNs that respond to both components A and B have arborizations in both cumulus and toroid. Thus these anatomically distinct MGC glomeruli are also functionally distinct with respect to their roles in processing of sex-pheromonal information and clearly exhibit narrow and specific molecular receptive ranges.

On the basis of their responses to antennal stimulation with pheromone components, uniglomerular MGC PNs have been classified in 2 broad categories: pheromone generalists and specialists^{34,35,37}. Pheromone generalists respond similarly to stimulation of either the component-A input channel or the component-B input channel and do not respond differently when the complete pheromone blend is presented to the antenna. In contrast, pheromone specialists can discriminate between antennal stimulation with component A and stimulation with component B. These PNs preserve information about individual key components of the species-specific blend. Our observations are consistent with behavioral studies and suggest that information about specific components of the blend, and not only the complete blend, is important to these animals. Another subset of pheromone-specialist PNs receives input from both component-A and component-B channels, but the physiological effects of the 2 inputs are opposite^{34,35,37}. Simultaneous antennal stimulation with both A and B elicits a mixed inhibitory and excitatory (-/+/-) response in these PNs. These PNs therefore can discriminate between the 2 inputs based upon how each affects spiking activity. Thus different output channels from the MGC convey infor-

mation about different aspects of the pheromonal stimulus. Such a study of odotopic organization of olfactory glomeruli depends on our ability to identify discrete glomeruli from animal to animal.

Higher pathways - After synaptic processing in the AL, information about odors is relayed to higher centers in the protocerebrum by way of the axons of AL PNs. We have begun to explore the physiological and morphological properties of neurons in the protocerebrum that respond to stimulation of the antennae with sex pheromone, host-plant odors, or their components^{40,41}. We find that many odor-responsive protocerebral neurons have arborizations in the lateral accessory lobe (LAL), which appears to be important for the processing of olfactory information. Different types of higher-order neurons link the 2 LALs to each other, to the ipsilateral superior protocerebrum, to the lateral protocerebrum (where axons of AL PNs terminate), and to the thoracic ganglia³²⁻³⁴. Thus, the LAL is interposed in the pathway of olfactory information flow from the AL through the lateral protocerebrum to descending neurons. Certain neurons associated with the LALs give dramatic, mixture-specific, long-lasting excitatory responses of a character not observed at the level of the AL. We have hypothesized that such long-lasting responses may prove to be important for generation of behavior that depends on specific mixtures of odorants

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PROBLEMS AND TRENDS OF AGRICULTURAL ENTOMOLOGY AT THE END OF THE 2ND MILLENNIUM

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Insect and acari are the main targets in agricultural entomology because they are pests of agricultural crops and vectors of transferable diseases (human, animals and plants). Insects are also beneficials (predators and parasitoids of pests); they play an important role in the food chain as a food; they are producers of food (honey) and other products producers (silk, wax), and extremely important as pollinators. Agriculture entomology of the end of the second millennium must face many problems which are connected with food production and deal with insects as competitors taking a large part of our yields.

Entomology is not an isolated science dealing only with all aspects regarding insects. It is strongly connected to other fields of our life, like demography, environment, transport, trade, tourism, food production, public health and others. The problems connected with entomology in the second millennium derived directly from overall human life and development in this period; some of them were created by human beings, others are created by insects themselves as a natural way of self defense. Problems that we created derived from our customs, behavior, economics, politics, effort to rule them according to our essential needs of providing food and fiber, and from health trends. Therefore, the main problems are pests and pest control for prevention of food losses and maximizing food supply, implementation of different control methods and their impact on environment and public health, especially extensive use of chemicals and development of new insecticides compatible with the environment, outbreak of indigenous pests, and introduction of new exotic pests to new areas.

Since human population will continue to grow, it is important to eliminate, as much as possible, factors limiting food production. Malnutrition is still the main problem in developing countries suffering from a limited food supply and from growing problems with pests and their control. According to the Food and Agriculture Organization (FAO) of the United Nations, 780 million people are suffering from hunger in developing countries only. Children are the majority with consistent undernourishment.

The growing demand for food depends on effective and reliable pest control, and chemical control is a very important part of it. Some environmentalists want to ban or at least to reduce the use of pesticides because of their influence on the environment, especially because of the uptake by crops and later on to be subsequently incorporated in the food chain, and because of contaminating soil, water, and having a detrimental influence on the ecosystem. On the other hand, people involved in agriculture claim that it is necessary to use those chemicals for getting a reasonable amount of yield and to reduce as much pest induced losses as possible. The most famous organochlorine insecticide DDT (Dichlorodiphenyltrichloroethane) was synthesized in 1873. In agriculture it was extremely effective against a number of pests devastating maize and potatoes. But the disadvantages

of its use soon became apparent, because it accumulates in fat body of mammals, it was responsible for the death of fish and birds.

The public, governments, health authorities, and environmental organizations are concerned about the tremendous use of insecticides, because of the apparent impact on health, environment and especially by contamination residues in water and surroundings, as well as the problems with applications when the toxic material is used in fields. Some insecticides have a chronic toxic effect on reproduction; they have mutagenic and oncogenic effects and cause long term damage to the nervous and immune system (Culliney et al, 1992).

In many countries the concern about health and environmental hazards led not only to banning some dangerous pesticides, but to serious cuts of all agriculture pesticides by legislation mandating the reduction of 50% or more of the total use by the year 2000. The three leading countries are Sweden, Denmark and the Netherlands. Two bills relating to pesticide use reduction were introduced to Congress in the USA in 1994, which included establishing and implementing pesticide use reduction (in selected ecosystems) and pursuing alternatives to chemical uses (Matteson, 1995). The main reasons for search and implementation of alternatives to pesticides as pest control methods are: (1) residual effect in agricultural products and the threat to the public health, (2) high cost of pesticides when the pest control rely entirely on expensive insecticides, (3) nonselectivity of some pesticides, (4) developing resistance in target pests, (5) effect on nontarget organisms, (6) outbreak of secondary pests, (7) high costs of developing new insecticides and obstacles with receiving a permit, (8) dangerous way of production, transport and application, (9) impact on environment (water, soil, air), (10) broad knowledge of pesticide science, high awareness and education of people to problems caused by insecticides, (11) high prices for non treated agricultural products, (12) antipesticides legislation and banning of some most effective insecticides, (13) political pressure (green peace movement, popular slogans in election companies) and finally, (14) the psychological impact. Problems connected with use of insecticides were the main forces to change the strategy of pest control and to implement Biological Control (BC), Integrated Pest Management (IPM) and other alternative, environmentally accepted methods, when they are economically motivated. The new products that expect to discharge or reduce chemical control are: bacterial insecticides, viruses, protozoan, nematodes, fungal insecticides, natural occurring pesticides (antibiotics and fermentation products), botanical pesticides (of plant origin), Insect Growth Regulators and Juvenile Hormones Analogs, semiochemicals (pheromones) and genetic engineering products (engineered baculoviruses and bacteria, transgenic plants producing toxins, genetically improved natural enemies).

In our millennium chemical control is still the basic method

for pest control, but because of all the mentioned problems the other alternatives are of great importance. The most important are Biological Control (BC) and Integrated Pest Management (IPM), although only about 5% of all pest problems are alternated by those methods. BC, according to Van Driesche and Bellows (1996) "is the use of parasitoid, predator, pathogen, antagonist, or competitor population to suppress a pest population, making it less abundant and thus less damaging than it would otherwise be. In the second millennium hundreds of examples of successful biological control are given by DeBach (1970), Huffaker (1971), Clausen (1978), Greathead and Greathead (1992). More than 220 examples of successful biological control involving 110 species of pests in 60 countries are given by Wilson (1970), more than 540 target insect pests in more than 1,200 introductions are included in biocontrol programs described by Greathead and Greathead (1992). From 860 releases in which 2,741 pest species were involved, 216 effective control cases with 393 parasitoid species were described (Van Driesche and Bellows, 1996).

BC however is not without problems. After a hundred years since the first successful use of biological control, fundamental questions such as how often, how many, and how and where the natural enemies should be released are still not known. In all cases the experimental design is critical and will require considerable time, effort and expense. These factors are probably the underlying causes of the lack of the research in this area (biological control) (Parella et al, 1992).

Several failures of unsuccessful introductions are given by many authors (Messenger, 1971; Hokkanen, 1985; Stiling, 1990). Fourteen different reasons why released natural enemies were deemed to fail (Stiling, 1993) are: (1) climate, (2) weather, (3) lack of synchronization, (4) wrong strain, (5) different habitat preferences, (6) host or prey refuge, (7) competition, (8) predation, (9) parasitism or hyperparasitism, (10) lack of alternative hosts, (11) lack of adult food, (12) low rate of increase, (13) too few releases, and (14) migration.

In the analysis of 148 cases of failure of introduction, the most common reasons were climate (24.3%), lack of alternative host (14.9%), and wrong strain. The other reasons did not reach 10%. Between them were the lack of synchronization (9.5%), hyperparasitism, parasitism (8.1%) and predation (6.8%) (Stiling, 1993). The mortality of natural enemies from sprays is caused by direct influence or by residual effect; other effect of insecticides are reduced fertility, shorter longevity, lack of hosts, repellency, low reproduction rates. Only few insecticides are considered to be harmless to natural enemies: pirimicarb, fenbutanine oxide, diflubenzuron, some plant derived insecticides, avermectin, narrow range oils, and systemic demeton and alocy-carb (Van Driesche and Bellows, 1996).

One of the important trends in second millennium is recognition of importance of Integrated Pest Management (IPM). Although IPM is described as an interdisciplinary approach to the solution of agricultural problems (Hoy and Herzog, 1985), it is based generally on biological control, carefully pest monitoring, developing thresholds followed by known control methods such as chemicals, which are used if it is necessary to avoid economic crop losses. The use of chemicals must be reduced to a necessary minimum, and include as much as possible, selective chemicals. Agrotechnical methods that are compatible with the environment are also a part of IPM and are incorporated in many ways. The implementation of these methods must be followed by excellent extension service, farmers education and of cooperation with responsible, in charge authorities.

As one of the solutions to replace the expensive and some-

times dangerous chemical control, was the attempt to use pathogens and nematodes, to find proper control agent and with all restrictions to find a practical use for them. This area has become an important and rapidly growing area of science. Spectacular success has already been achieved by tide cooperation of entomologists with virologists, microbiologists, parasitologists and scientists from other related fields. Insect pathogens are environmentally safe, selective, some of them once introduced provide sufficient pest control for an extended period, some of them have even competitive prices with chemical insecticides. "Successful commercial development of pathogens augmentative biological control involves: agent selection (to obtain the best species and strains for the target pest); development of cost-effective methods for mass rearing; effective methods for storage and shipping of the agent; creation of formulation to protect and deliver the agent to the target pest's location; field testing of the product's efficacy and method for its application; economic factors affecting product development and markets; and demonstration of product to man and the environment" (Van Driesche and Bellows, 1996). The following groups are used: viruses, bacteria, fungi, protozoan, and nematodes. But those products are as well not free from general problems of chemical insecticides and recently there are reports on resistance developed in insect against some granulosis virus and *Bacillus thuringiensis*.

Insect Growth Regulators were accidentally discovered in 1970 by Philip-Duphar scientists in The Netherlands (Van Daalen et al, 1972; Wellinga et al, 1972 a,b). They are divided into two major groups: (1) Chitin Synthesis Inhibitors (CSI's) and (2) Juvenile Hormone Analogs (JHA's) (Miyamoto et al, 1993). Those CSI's are developing molt disruption of lepidopterous larvae, however they may have adverse effects on beneficial insects. Additionally to larvicidal effect, in some cases they show ovicidal and sterilizing activities, suppress fecundity and demonstrate contact toxicity (Arambourg et al, 1977; Asher and Nemny, 1974). Juvenile Hormone Analogs interfere with insect metamorphic changes.

The different groups of those insecticides are now incorporated in many programs of IPM, but not surprisingly they are not free from the problems of chemical insecticides. Resistance is found more and more among them. Although the discovery of CSI's led to extensive research in the field of insect cuticle biochemistry and chitin synthesis, hundreds of chemicals were screened and found to be potent inhibitors; many more studies are needed to evaluate those products, especially with regard to environmental impact. Without intensive studies of resistance and establishing IPM strategies, those insecticides will suffer from all problems of chemical commercial insecticides.

The use of semiochemicals was a significant development in agricultural entomology. Semiochemicals are compounds that are emitted or displayed by one organism and that affects the behavior of another organism (Rigway et al, 1990). Chemical communication is important in life cycles of many insects, especially for location and mates and food. They are widely incorporated in many IPM programs and used in two ways: by monitoring pest population and by preventing insect caused damage. The most common semiochemicals are pheromones, followed by antifeedants and repellent compounds (Bjostad et al, 1993). The principal value of monitoring is to reduce the amount of insecticides but at the same time maintain acceptable insect control. Early detection of pests, the presence or absence, has a crucial role in decision making regarding the insecticide application.

The most common use of semiochemicals is in behavior disruption, on which the technique of the placement of many

sources of pheromones placed in the field caused "confusion" of the males. The males are unable to locate the female mate and the large amount of synthetic pheromones in the field confuse them. Another technique developed is mass trapping where the male population is significantly reduced and the unmated population of females fail to produce offspring. Another trend in semiochemical research and development is evaluation of the influence on natural enemies. Some of the parasitoid wasps are attracted to pheromones of their host and increase in such a way the search ability of the parasitoid to find the target pest.

Important steps in development of pheromone research include practical use for direct control of pest populations and for integrated control in IPM programs together with agricultural methods, biological control agents, and insecticide, and such interaction can maximize the advantages of all of them. The advantages of using pheromones are: their selectivity, they are specific for each pest species, they are degradable - therefore there is no residual effect, non toxic, do not interfere with biological equilibrium by absence influence on beneficial insects, and the high cost of production is balanced by the small amount usually used. The research that will provide any understanding of the communication system, will be a result of close cooperation of entomologist, biochemist, physiologist, behaviorist, molecular biologist, and will lead to new, more sophisticated pest control and plant protection.

To find insecticides more compatible with IPM and environmental requirements, in the last three decades interest was revived for insecticides originating from plants. Plants are a rich source of phytochemicals and until now about 10,000 such chemicals have been isolated; the estimated number is closer to 500,000. Natural pyrethrins, nicotine and rotenone have been in use for a long time. The most prominent are now the Neem products from leaves and seeds of Neem trees, called Indian lilac, *Azadirachta indica* (= *Melia azadirachta*) or from related species. Despite selectivity, there are about 200 species controlled by Neem products and, until recently, there is no reported case of resistance.

In 1976 in a culture of *Streptomyces avermitilis* (Burg), collected in soil samples in Japan, scientists discovered a complex of eight closely related natural products which were insecticidal in nature and named avermectins. They have insecticidal activity against many important insect pests and are more effective against many mite species, but resistance was already found to this product in mites (Hoy and Conley, 1987). These products are candidates "for further development representing linkage of many scientific disciplines spanning natural products, chemistry, organic, synthetic and biochemistry with the applied investigators in agriculture, human and animal health" (Bowers, 1989).

The most recent development in plant protection connected with entomological research and in the beginning of implementation is genetic engineering using molecular methods. To improve the performance of *Bt* and viruses different methods are used. To overcome the limited host range of *Bt* several methods were employed to combine different strains in one product. Recently new genetic engineering methods are used to increase the ability of killing pests by viruses by inserting specific genes into baculoviral genome. Baculoviruses are used as an excellent expression vectors for basic and applied research, because they have easily modified genomes, strong promoters to drive foreign gene expression and markers for the detection of foreign gene insertion. They are widely used as vectors for foreign gene expression in insect cells by replacement of the polyhedron gene with a foreign gene (Maeda and Hammock, 1993).

Peptides and polypeptides, ectosteroids and juvenile hormone

analogues of insect and noninsect origin can exhibit insecticidal effects without affecting other organisms (including vertebrates). The following are the categories of candidate genes for construction of recombinant baculoviruses for insect pest control: enzymes and enzyme inhibitors, neuropeptides, venom toxins (of scorpion, mites, spiders and insect paralytic wasp) and *Bt* toxins (Maeda and Hammock, 1993).

Other genetically engineered products include plants producing insecticide to repel or kill phytophagous insects. They can be genetically manipulated and produce a higher level of natural insecticide (introduction of *Bt* toxin). Generally, the plants in which genetic material from other species was inserted by biotechnology are called transgenic plants and were developed for major crops of the world (Jenkins, 1993). This genetic engineering technology will have an important impact on the pest management strategies in the major crops (potatoes, corn, cotton, etc.) and will be implemented not only on an economical basis, but with their compatibility with other practices as well, like biological or IPM control methods. Those transgenic plants are produced on a commercial scale and will probably suffer from appearance of resistance in the pest attacking those plants. Recently after more than 20 years of using commercial *Bt* preparations, resistance to *Bt* is reported not only in laboratory experiments but in the field as well (Tabashnik, 1994).

The next step in genetic engineering is genetically improved strains of beneficial insect, not only by selection but by transferring to them pesticide resistant genes by recombinant DNA techniques. Recombinant DNA (rDNA) techniques to develop transgenic beneficial arthropods have been developed recently and are useful when the natural enemy is effective except for some limiting factors and the limiting trait primary is influenced by a single major gene (Hoy, 1993). Those new techniques offer new opportunities for pest management but created as well many problems connected with many still unknown results. The greater the genetic novelty, the greater are the possibilities for surprising results (Williamson, 1992). Therefore, for the first release it may be proper to choose a relatively risk-free beneficial. Such a candidate becomes a predatory phytoseiid mite *Metaseiulus occidentalis* a transgenic strain developed by inserting gene for organophosphorous (OP) resistance, and in December 1995, Marjorie A. Hoy (University of Florida) asked USDA for permission for first outdoor releases of this altered beneficial, and in March 1996 the first experiments were performed. It may take 5-10 years of evaluating small-scale releases before permanent releases will be permitted.

The high trade movement of all kinds of merchandise, lack of effective quarantines, the boom of tourism and other kinds of people movement (like emigration, family reunions, transfers, search for work), and related exchange of products of different agricultural origin, unintended introduction or intended smuggling of plants and plant origin products, favor the spread of every exotic pest, which will become a pandemic pest and will not be any more limited to certain areas of their origin. Samples include introduced lately insects that become world-wide pests, such as the most famous western flower thrips, *Frankliniella occidentalis* and the latest citrus pest, the citrus leafminer, *Phyllocnistis citrella*. A new threat for the citrus industry arrived in Florida in 1995, with the detection of a tristeza virus carrier, the brown citrus aphid, *Toxoptera citricida*. It is expected that this aphid will infest most of the citrus growing area of Florida at the end of 1996.

It is only a matter of time, suitable environment including proper host, adaptation and we have a new pest in a new part of the world. Only in Florida is it estimated that approximately 12

exotic species are introduced annually (Capinera et al, 1994). From a list of 343 scale insects recorded in Italy from the 1980s until 1993, 44 species were introduced. Those species are native *inter alia*, to Australia, Eastern Asia, China, Central America, Northern America and South Africa (Longo et al, 1995). Taking into consideration only Coccinac, in The Netherlands during 1950-1994, 143 species were intercepted (Jansen, 1995).

No doubt that the best way to deal with introduced new pests is prevention, but it is impossible to seal hermetically the borders and the in gates of entrance. However, it is possible to reduce the invasion of exotic pests by enforcement of effective quarantine regulations. This will be provided by more manpower on the border stations, and more effective inspection of agricultural commodities and through education, proper explanation of the danger of such introductions and proper information on consequences of uncontrolled plant material movement. Once introduced, pests are likely to establish and can be controlled by eradication or by introducing as soon as possible natural enemies from the country of pest origin (biological control). Eradication is expensive, sometimes ineffective and disrupts the biological balance between exotic natural enemies of other pests in the same environment and as a result not successful. When natural enemies are introduced, it must be made as soon as possible to avoid the spread and establishment of the pest in large areas. In some countries, governments instead support the import of natural enemies by giving immediately financial support and import permits, sometimes delay by cumbersome restrictions, natural enemy introduction, mass breeding and field releases. The concern about introduction of natural enemies is sometimes irrational and the danger to endemic fauna is overestimated.

The next vital problem at the end of the present millennium is education of a new generation of entomologists. The progress in developing reliable plant protection methods which will include all the above described methods, in different fields of entomology, will depend on funds that governments, private sectors, scientific funds and growers' associations can provide to scientists and extension services. This will support the basic and applied research, developing new, more expensive technologies and attract a young generation of scientists dedicated to different fields of entomology. In entomology, one of the problems was lack of essential support for research in systematics. Oliver (1988) wrote that a purpose of his article is "to alert the entire scientific community to the crisis in systematics in all its manifestations, including identification service and evolutionary history, and scientists from other disciplines and administrators must understand the practical and immediate role that systematics plays in many areas." Recently, the authorities appear to understand this warning, and the pressure from scientists that recognize the gap in this field, that created problems with identification of insects and especially pests, lead to recruitment of more young entomologists being employed in this field.

Extension service serves as a messenger from research to producers. The traditional extension research model has been historically defined by five stages: (1) extension specialist listening to producers, (2) reporting problems to researchers, (3) helping solve the problem, (4) conveying the solutions back to producers, and (5) implementing the solutions (Gray and Edwards, 1993). Additionally, the extension service must provide all the available information regarding specific pest problems. Since more and more people involved in agricultural production are highly educated, and the modern agriculture requires basic skills as well, the extension services are more important and expected to be more sophisticated. From an extension service officer, who provided all needed information, we went up to a new era of

extension when the well educated officer must not only supply the necessary information and give answers for many questions to avoid or reduce the level of economic injury, but must also adapt this information to local conditions (like climate, soil, surroundings) taking into consideration different pest appearance and their natural enemies. In addition, he must be the messenger who delivers all the new information achieved by scientists to practical, large-scale use. So much depends on extension services if the tremendous available knowledge supplied by research will find its way to the field, and the greatest challenge will be to go from high technologies to extensive practical implementations.

The new generation of educated producers and availability of needed information through the electronic media, may be probably less dependant on extension services. Therefore there must be a strong partnership between scientists, extension service and producers.

There are now a wide range of databases and a part of them are relevant to plant protection and pest management. Hamilton (1991) listed 14 such databases, connected with pest management, the largest ones are BIOSIS PREVIEW, CAB ABSTRACTS, AGRICOLA, AGRIS, CSA LIFE SCIENCE COLLECTION and some of them highly specialized in this field, PEST MANAGEMENT RESEARCH SYSTEM, PEST-DOC. They are of different sizes ranging from 750 records to 6,156,000 records (BIOSIS PREVIEW) with annual growth rate of 360,000. The time span started of some of them from 1968 or even less and this created problems specially for young scientists that the previous data must be still gathered by old methods.

VanDyk (1995) provides us with information regarding the use of the Internet as entomologists. The Internet provides useful tools including e-mail (electronic mail), mailing lists, Uniset, Gopher and World-Wide Web. E-mail is the most useful tool which allows rapid communication with anyone who has access and an account. It is extremely fast, it is sent, sorted and received by computer, without paper or envelope, and allowed to get an immediate answer. Electronic mailing lists are a forum for discussion and announcements to everyone on the list. Uniset and Gopher are other programs providing communication and exchange of information. The World-Wide Web is the most exciting way to exchange information and based on text with links and links consist of words that will lead you to information related to links. Information is not limited to text but pictures, sound and movies are provided.

In the future many (if not all) journals will be published electronically. The first entomological journal is the Florida Entomologist, available on Internet since vol. 77 of 1994.

The end of the second millennium was characterized by tremendous cooperation of scientists from many disciplines of basic and applied entomology. By using new developed technologies, such as tissue culture, *in vitro* culture of pathogens, developing transparent and scanning electron microscopes, chemical and biochemical methods for detection of new pesticides, discovery of the mode of action, use of irradiation techniques, employee semiochemicals and disruption of physiological processes, genetic engineering and genetic improvement etc. will cause an "emergence of cross disciplinary insect scientists motivated by both fundamental and applied perspectives and fueled by the national (and international) need for selective, environmentally pacific methods of pest and disease control promises to enhance the stature and visibility of our science of entomology for the next hundred years" (Bowers, 1989).

With all this tremendous progress in the mentioned technologies and new, more and more sophisticated methods, will not be

a substitute for human brain, eyes, intelligence, ability of observation and possibility of drawing right conclusions. Of many subjects that I tried to cover in this paper, probably there are a lot more important developments in agriculture entomology that are

in the meantime, small discoveries, but they are bricks in the hugh building of plant protection research and they will find their expression and will influence the future research and practical applications.

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ROLE OF SALIVA IN BLOOD FEEDING BY ARTHROPODS: DIVERSITY AND REDUNDANCY

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It is very well known to entomologists that insects represent a very large and diverse group of animals. Indeed the sheer number of insect species supersedes that of all other land animals and plants taken together. Additionally, insects have evolved and radiated in a way to exploit all forms of food and shelter, in many cases many different species providing many different solutions to the same environmental, physiological or biochemical problem. The diversity of solutions insects found to their common life problems thus provides the biologist with a rich source to understand life in its many facets.

Among the many different food sources insects evolved to exploit, blood from vertebrate animals, in particular, became the sole source of food for many insects, or the source of food for the adult female insect in her search for a protein rich meal to provide for egg development. Thus, at least 12 insect families evolved independently to hematophagy, consisting of 402 genera and 13,795 species. If we lump together also the tick cousins, which medical entomologists adopted in their practical wisdom, we add another 800 species of blood sucking arthropods¹⁹. If one realizes that all land vertebrates from turtles to mammals have a total of 18,100 species⁹, there exists about one blood sucker arthropod species for each land vertebrate species. Considering that mammals evolved in the past 60 million years, and that even insects within the same family can be more distant than 100 million years, than it is apparent that a large number of species have undergone convergent evolution to exploit this unique food source.

Among the unique problems insects and ticks had to adapt themselves were: (1) How to find a host? (2) How to take blood out? (3) How to digest and cope nutritionally with this unique meal? Many of the interesting research going on vector biochemistry and physiology now addresses these questions. This short review will address the problem blood sucking arthropods face when taking blood from their hosts.

The problems associated with stealing blood from vertebrates - When attempting to take a blood meal, insects and ticks face problems of both mechanical and physiological nature. The mechanical aspects of blood feeding were solved in two basic types, either by piercing and sucking mouthparts (leading mostly to feeding from within vessels), or with cutting mouthparts, where feeding is done from a hemorrhagic pool. In any case, successful blood suckers need to keep the blood flowing during the feeding process and against this goal there occurs the vertebrate hemostatic system, which prevents blood flow from injured vessels by a very redundant and sophisticated physiological system consisting of platelet aggregation, vasoconstriction and blood coagulation. Insects and ticks have thus evolved a very amazing array of pharmacological antihemostatic substances which makes them true pharmacologists every time they feed, if by pharmacologist we understand as someone that makes a liv-

ing by changing someone's else physiology¹⁹.

In the remaining of this paper the host hemostasis will be reviewed, as well as the several mechanism hematophagous arthropods found to disarm their host's hemostasis. Emphasis will be given to show that when common things shows up, they are actually different.

The vertebrate hemostasis - To have in perspective the complexity of the task of counteracting the vertebrate hemostatic system, it is useful to realize that blood clotting, platelet aggregation and vasoconstriction mechanisms are redundant themselves: blood coagulation occurs through two pathways, the intrinsic and the extrinsic, both of which, through independent mechanisms, activate Factor X to Factor Xa. The intrinsic pathway is triggered by the exposure of blood to subendothelial components, such as collagen. This can activate plasma Factor XII, leading to a number of reactions ultimately activating Factor X. The extrinsic pathway is triggered by the release of tissue thromboplastin from injured cells, which also ultimately leads to activation of Factor X. Factor Xa converts prothrombin to thrombin, the enzyme that produces fibrin from fibrinogen. Platelets can aggregate from at least 3 pathways, induced by ADP, thrombin, or collagen (which triggers platelet synthesis of the eicosanoid thromboxane A₂). Although each of these mediators may potentiate the other's action, each individually can give a full aggregatory response if the proper dose is present. Finally, vasoconstriction of venules and arterioles can be promoted by serotonin and thromboxane A₂ released by aggregating platelets, and by other mediators released by local neural reflex, or other inflammatory cells, such as neutrophils and mast cells. Accordingly, blood sucking arthropods have evolved an array of different anticoagulants, anti-platelet substances, and vasodilators^{12,18,19}.

Anticoagulants - Since the beginning of the century it is known that saliva of blood sucking arthropods contain anticoagulant properties⁷. Indeed, at that time it was believed that only coagulation accounted for hemostasis, despite the observations of Bizzozero at the end of the last century, implicating platelets in hemostasis³. In the last decade a number of anticoagulants have been characterized molecularly, indicating a vast array of different molecules acting against different factors of blood coagulation. It is interesting that there is a concentration of anticoagulant factors against Factor Xa and thrombin, the two final steps of the coagulation cascade. Unique polypeptides affecting the stability of the Xase complex have being discovered in *Rhodnius* and *Cimex*²³, Valenzuela and Ribeiro, in press). Such anticoagulants do not act by antagonizing enzymes, but rather disrupt the complex formed by phospholipids, Ca²⁺, Factor VIII and Factor IXa, this complex being responsible for activating Factor X to Xa. It is also interesting that blackflies, which feed from hemorrhagic pools and thus face a double threat from coag-

ulation, have developed a cocktail of 3 different anticoagulants, an anti-Xa, an anti-thrombin and an anti-Factor VII^{1,2,11}. Ticks, who also feed from hemorrhagic pools, have developed anti-tissue multiple anticoagulant cocktails¹⁰, and it may be possible that such mixtures of anticoagulants will also be found in other hemorrhagic pool feeders.

Anti platelets - Platelet aggregation represents the most immediate reaction against blood loss, indeed being sufficient to arrest blood flow from small vessels in the absence of a functional blood clotting system¹⁵. Not surprisingly, most hematophagous arthropods so far studied have abundant salivary anti-platelet activities. Among the anti-platelet compounds, the enzyme activity ATP-diphosphohydrolase (apyrase) has been a common finding. The salivary apyrase of the mosquito *Aedes aegypti* has been purified and cloned, and shown to belong to the family of 5'-nucleotidases⁶. Other apyrases from hemiptera are being cloned (Champagne, unpublished), and appear to have originated from an altogether different family of proteins. A picture is emerging indicating that even the common finding of salivary apyrase in blood sucking arthropods derives from convergent evolution.

In addition to apyrase, ticks have prostaglandins that have antiplatelet activity, and more recently a variety of unrelated peptides that target different sites in the process of platelet aggregation were discovered in deer flies and triatomine bugs¹⁹. The triatomine bug *Triatoma pallidipennis* contains at least two homologous proteins with sequence similarity to apolipoprotein D, named pallidipin¹⁶ and triabin¹⁷, which inhibit collagen induced platelet aggregation and thrombin, respectively. This shows how a possible scenario of gene duplication may have lead to different functions in homologous molecules.

Vasodilators - Salivary vasodilators are widely found in blood sucking arthropods, and these are thought to help feeding by counteracting the vasoconstrictors released by aggregating platelets, or to increase the skin blood flow and thus the supply of food to the feeding insect. Perhaps because of the diversity in the species background from which blood feeding evolved, a corresponding diversity in vasodilators exists. Thus, prostaglandins, peptides, nitrovasodilators and enzymes destroying vasoconstrictor amines have been found in the saliva or salivary gland homogenates of blood sucking arthropods.

An interesting finding in blood sucking hemiptera (*Rhodnius* and *Cimex*) was a salivary nitrovasodilator that consists of the gaseous and reactive substance nitric oxide (NO) associated with a myoglobin-like molecule that was named nitrophorin^{4,20,25}.

Rhodnius prolixus has 4 such nitrophorins, all now cloned (Champagne, unpublished), and *Cimex lectularius* has one such molecule, also cloned (Valenzuela, unpublished). *Cimex* and *Rhodnius* nitrophorins are completely unrelated molecules, and are thus products of convergent evolution serving the same purpose, that of carrying the vasodilatory NO gas to our skin, so the bugs can feed faster.

The sand fly *Lutzomyia longipalpis* contains a novel vasodilatory peptide that has been characterized as the most potent and persistent vasodilator known. This peptide of approximate MW of 7,000d, is now sequenced and synthesized^{13,14}. The vasodilator may be responsible for the immunosuppressive effect of sandfly saliva leading to enhancement of *Leishmania* infection²⁴. Interestingly, Warburg and cols²⁶ noticed that there is a very large variation, both in amount of vasodilator and in peptide sequence, among different geographical isolates of the fly *Lutzomyia longipalpis*. These differences are also correlated with differences in the epidemiology of clinical manifestations of visceral leishmaniasis in Central and South America.

Recently the salivary vasodilators of *Aedes aegypti* have been sequenced and shown to be octapeptides belonging to the family of tachykinins⁵. Similar vasodilator was found in *Ae. triseriatus*²², but *Anopheles* mosquitoes possess a peroxidase with activity against vasoconstrictor amines²¹, and do not possess tachykinins. The totally different process these two mosquitoes found to increase skin blood flow is perhaps not surprising if we take in consideration that Culicines and Anophelines have separated 120-150 million years ago and mammals have irradiated about 60 million years ago.

Currently, vasodilators from black flies have been purified⁸ and cloned (Cupp, unpublished), and represent a totally new family of vasodilators (Marydillan).

Conclusion - With over 14,000 species of blood sucking arthropods in over 400 genera, the complexity of the hemostatic vertebrate system lead blood sucking arthropods to evolve a not less array of pharmacologically active substances that we are now only beginning to comprehend. Even when similar things appear, as in the case of the apyrases, or nitrophorins, they may be non homologous and thus a product of convergent evolution. When they are homologous, as in the case of the sand fly vasodilator Maxidilan, a large diversity may exist that represents a naturally occurring site-direct mutagenesis-type of work that may be useful for structure/function studies. It will take some time to learn what these blood suckers have to teach us, but it will be an interesting and meaningful endeavor.

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PATTERN FORMATION IN INSECT EMBRYOGENESIS: THE EVOLUTION OF CONCEPTS AND MECHANISMS

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This lecture, which is to acknowledge an award*, will review two interrelated topics. Both concern the mechanisms of embryonic pattern formation, defined as the manifold processes that transform the simple spatial structure of the egg cell into the highly complex three-dimensional organization of the future body. This transformation, repeated stereotypically in each new generation, is one of the most characteristic properties of living beings. Our insights into the mechanisms that accomplish it has dramatically increased in recent years, primarily by research on insect embryogenesis.

Of the two evolutions addressed in the title, the first is part of the history of biology. It concerns the major conceptual steps by which insect embryologists have reached their present knowledge and insights. The second (treated rather briefly) concerns a basic aspect of biological evolution, namely the progressive modification of embryonic patterning mechanisms while crucial molecules involved were strongly conserved. Two sets of such molecules have recently provoked novel and far-reaching conclusions concerning the course of metazoan evolution at large, and these will be briefly outlined as well.

Before embarking on either kind of evolution, the diversity of early embryonic patterning in insects needs to be outlined (Krause, 1939; see Sander, 1976)**. In “short germ” development, the as yet unsegmented embryonic rudiment (or “germ anlage”) is short by comparison with the egg cell, and gives rise to the segmented germ band by “budding”, i.e. by generating the body segments sequentially from anterior to posterior. In “long germ” development, a store of organelles etc. provided by the nurse cells permits the rapid development of a large germ anlage (often as long as the egg itself) which then subdivides more or less simultaneously into the full number of segments. In many insect groups, traits of both these segmentation modes are combined: the major part of the germ anlage yields some anterior segments by (nearly) simultaneous subdivision whereas the remaining segments are added sequentially. This is the “semi-long” or “intermediate” type of patterning. Besides segmentation as such, its relative timing with reference to gastrulation may serve to define the three (or more?) insect germ types (Patel). With respect to biological evolution, long germ development no doubt is a derived mode while opinions differ on the mode ancestral for insects (short or intermediate germ?).

As for mechanisms patterning animal embryos, the modern view combines traits of two philosophical concepts, namely epigenesis and preformation. These ancient terms were revived early in our century (mainly by work on sea urchin embryogenesis), but with some change of meaning: preformation prevails in the genes, but their linear information is transposed into the complex three-dimensional structure of the body by epigenesis,

now defined as the intricate interplay of relevant genes with each other and with some simple spatial cues conferred on the egg cytoplasm during oogenesis.

Insect development has influenced concepts of embryonic patterning since the beginnings of mechanistic biology in the 18th century. The first observations, however, seemed to support a concept which denies any *de novo* patterning, namely embryonic preformation. Noting that newly born viviparous aphids can encase a complete larval body representing the next generation, Charles Bonnet claimed the “*préexistence en miniature*” of the offspring within the mother, and postulated the bodily “*emboîtement*” (encasement) of all future generations.

However, progress in microscopy revealed that the egg (or spermatozoon for that matter) does not contain the body *en miniature* to begin with, and the concept of *emboîtement* became discredited because of the minuscule dimensions implied for the preformed bodies of distant generations. The conceptually simplest way for bypassing these obstacles while maintaining mechanistic causality was “neo-preformation”. It replaced the various parts of the (no longer pre-existent) body with a preformed pattern of invisible “determinants” in the egg, each determinant directing the development of the respective body part in its place.

Despite much evidence to the contrary (e.g. in sea urchin embryogenesis), variants of this static “mosaic egg” concept have lingered on in some minds well into our century - and nowhere longer than among *Drosophila* researchers (e.g. Waddington, 1956) where they ultimately took the guise of pre-localized determinants for individual imaginal discs. This idea may have been encouraged by Ernst Hadorn’s pioneering work on imaginal disc determination (see Garen, 1992) and by the demonstration of prelocalized germ line determinants (see Sander, 1984) which lent themselves as a model for putative determinants in other parts of the egg cell. However, most proponents of this illicit extrapolation knew that it would not solve the problem of *de novo* generation of spatial complexity, but merely shift the burden from embryogenesis to oogenesis which accordingly - and vainly - began to be screened for appropriate mutants (see Garen, 1992).

Factual evidence against the putative mosaic of ooplasmic determinants was first obtained for intermediate and short germ development, mainly by duplications and complete twinning that had been induced in these forms experimentally (see Seidel, 1936). Meanwhile, a more dynamical alternative to the mosaic egg concept had been established for some other animal groups. Here embryonic patterning was ascribed to inductive signals (of unknown constitution) spreading from a morphogenetic centre - the “centre of first differentiation” in the sea urchin embryo or

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** References preceded by “see” refer to reviews.

the “organising centre” in amphibians (see Sander, 1996).

Seidel proposed a formally similar concept for the insect embryo, but - for reasons quite plausible at the time - he entrusted the wrong centre with generating embryonic complexity. He and his students had discovered that the segment boundaries become visible one after another even in some long germ species, for instance the honeybee. Like some other visible changes (notably gastrulation), this subdivision was found to begin somewhere in the middle of the future pattern, at a location named *Differenzierungszentrum* (differentiation centre). However, Seidel later on went beyond this descriptive concept by crediting the differentiation centre with a physiological task, namely the specification of individual segments that were about to make their appearance anterior and posterior to centre (see Seidel, 1936). This conceptual switch from the “morphological” to the “physiological” differentiation centre was supported by rather complex evidence that became devaluated by subsequent findings (see below). Seidel’s assumption that the course of visible segmentation is strictly linked to segment specification clearly does not hold for silkworm embryogenesis: here the sequential appearance of visible segment boundaries subdividing the germ anlage is preceded by simultaneous segment delineation at the molecular level (Kraft and Jäckle, 1994). Moreover, the data that Seidel quoted in support of his concept are open to alternative explanations based on the progressive commitment of cells to their specific fates (see Sander, 1976).

Doubts concerning the “physiological differentiation centre” were triggered by the first embryonic transplantations (or rather intra-embryonic translocations) performed in insects, using the leafhopper *Euscelis* (Sander, 1960). Together with a large body of other experimental data, the translocation experiments showed that material from the posterior egg pole exerts a long-range effect on patterning over a substantial part of the egg cell: a translocated “posterior centre” did not only influence the character of whole series of segments, but also their polarity - irrespective of whether or not the series contained the differentiation centre. Moreover, these experiments indicated that the effects of the posterior centre are being antagonized by some influence from the more anterior parts of the egg cell which - by comparison with data subsequently obtained in dipterans - might be called an anterior centre*.

The leafhopper experiments (Sander, 1960) also yielded the first concept of how, in insects, an embryonic “centre” could generate a pattern of different segments - namely via a morphogenetic gradient (Sander, 1960). With increasing distance from the centre, some substance(s) produced there would decrease in concentration, and the different concentrations prevailing along this gradient might locally trigger different genes directing the development of different segments. In order to account for certain experimental results, the additional assumption was required that in the leafhopper both centres generate gradients that can interact in some way.

This formal concept of “polar centres” establishing antagonistic gradients embodies several traits meanwhile documented down to the molecular level in *Drosophila* (see below). However, in hindsight the original concept (Sander 1960) was much too simplistic. Moreover, even several data from the leafhopper itself (e.g. Vogel, 1978) required some conceptual amendment, mainly concerning the abdominal segments which the original model (Sander, 1960) indeed had failed to specify in detail. This may mean that the abdomen derives only its polarity

from the gradient system whereas its individual segments form in the sequential “budding” mode - as *per definitionem* required for the intermediate germ type. A more profound revision might be invited by novel findings on the posterior morphogenetic gradient in *Drosophila* (see below).

The concept of morphogenetic centres at the embryo’s poles was soon to receive support from experiments involving a variety of other insect species (see Sander, 1976), especially beetles and lower dipterans (Nematocera). In the latter (midges etc.), each end of the egg cell, anterior as well as posterior, can experimentally be caused to adopt the patterning program of the other, whereby “double abdomens” and “double cephalons” result (Yajima, 1960). This proof of polar influences was followed by the demonstration that newly laid chironomid eggs harbour at the anterior oocyte pole some kind of RNA serving as “anterior determinants” of pattern formation (Kandler- Singer and Kalthoff, 1976).

In higher dipterans (Brachycera, flies) the first evidence for pattern specification being influenced by both egg poles came from ligation experiments in blowfly embryos (Herth and Sander, 1973); the segment patterns obtained were definitely incompatible, on the other hand, with the “mosaic egg” hypothesis. Further evidence for polar influences on patterning came from the mutant “double posterior” or “double anterior” embryos (resembling the chironomid “double monsters”) that were found in maternal effect mutants of *Drosophila* (Bull, 1966; Lohs-Schardin and Sander, 1976, for details see Lohs-Schardin, 1982).

These *Drosophila* mutants heralded the dawn of a new epoch of developmental biology, characterized by large-scale research on animal species that are amenable to genetics and hence to ever more sophisticated molecular methods. This epoch was initiated by the meticulous analysis of *Drosophila* patterning mutants by Lewis (1978) and Nüsslein-Volhard and Wieschaus (1980) recently acknowledged by a Nobel prize. The data accumulated since their pioneering publications are so overwhelming in both quantity and complexity that only a few can be outlined here; for more detailed information see e.g. StJohnston and Nüsslein-Volhard (1992), Bate and Martinez- Arias (1993), and Carroll (1995).

The molecular patterning concept that has emerged is a hierarchical cascade of interacting regulatory genes, provided with polarity by simple spatial cues from the egg poles*. The successive levels of the cascade produce increasingly complex spatial patterns of gene expression even before any segments become visible. The first step in this process is based on the *bicoid* mRNA localized at the anterior pole of the *Drosophila* egg cell. This anterior “organising centre” yields a gradient of the *bicoid* protein serving two functions. (1) It acts as a morphogenetic gradient that specifies the anterior body segments by locally activating the appropriate genes of the second level. (2) It shapes a posterior morphogenetic gradient specifying the posterior segments. This gradient consists of the *caudal* protein and arises by translational control: the *bicoid* protein is capable of inhibiting translation from the evenly distributed *caudal* mRNA and this leads to a gradient of nascent *caudal* protein which has its maximum where *bicoid* concentration is minimal, i.e. posteriorly (Rivero-Pomar et al, 1996).

The morphogenetic function of the *caudal* gradient remained hidden for some time because of an additional complication that can be linked to a step in the evolution of insect patterning

* Note that, for brevity, these and all subsequent considerations will be restricted to longitudinal patterning.

mechanisms, namely the transition from intermediate to long germ development. The posterior centre, apart from inducing the germ line, produces a protein encoded by the *nanos* gene. The *nanos* protein serves to repress the synthesis of yet another protein (encoded by the gene *hunchback*) that in turn would block posterior patterning based on the *caudal* gradient (as described above). This complication may have arisen from a short or intermediate germ ancestor where the repression of *hunchback* was a precondition for the sequential formation of the posterior segments. If so, this repression had to be maintained during transition to the long germ mode since otherwise the abdomen would have been lost in this transition. It might be interesting to check whether the leafhopper data can be reinterpreted on this basis.

These considerations have implicitly paved the way from the evolution of concepts to that of patterning mechanisms. As a crucial component of these mechanisms, the HOX complex of "positional" genes will be considered here*. In *Drosophila*, the unique character of each segment (its "identity") depends on the activities of some members from a set of master genes (or selector genes) known as homeotic genes. Mutants affecting their function (see Lewis, 1978, Carroll, 1995) lead to "homeotic" transformations of the a restricted body region, causing for instance the third thoracic segment to look like a copy of the second, or the antennae like legs. The activation of these genes at the proper location in the early embryo is based on the primary bipolar gradient system that also directs segmentation. On the chromosome, the homeotic genes are arranged as a linear cluster named the HOX complex (split in two parts in *Drosophila*). In this complex, the individual genes - together with the nucleotide sequences needed for their proper spatial regulation - are lined up in a sequence "co-linear" to that of the body segments whose identity they influence most strongly.

The HOX complex was subsequently found to also occur in many other animal groups, and thus must have been highly conserved during evolution (see Akam et al, 1994, Carroll, 1995). The individual HOX genes mediate the position-dependent activation of different "batteries" of target genes. The latter then shape those organs that are appropriate for the respective position in different body plans. As shown by its taxonomic distribution, the HOX complex obviously antedates not only the divergence of the arthropods from related phyla, but even the divergence of proto- and deuterostomians. Consequently the HOX complex may provide a criterion by which all metazoa might be defined as a group (the "zootype" of Slack et al, 1993).

Another highly conserved system of "positional" genes seems now to emerge for the dorso-ventral axis. This system may serve

for inferring the way by which the earliest deuterostomians derived from protostomian ancestors. Contrary to some more sophisticated concepts, this transition must have occurred by a bodily rotation around the animal's longitudinal axis, i.e. by using the former dorsal side as the new ventral side. The argument rests on the fact that in insects and amphibians the dorso-ventral axis is being defined by two groups of mutually inhibitive genes - but the genes defining dorsal in insects are defining ventral in amphibians (Arendt and Nübler-Jung, 1994, De Robertis and Sasai, 1996).

These examples document an amazing conservation of (positional) genes during the profound evolutionary changes in body shape that enable us to distinguish vertebrates from insects. To contrast this molecular conservation, some examples of molecular innovation during evolution will now be outlined to close with. Gene duplication followed by modification of (at least) one descendant gene is an accepted mode of genetic innovation, but insects demonstrate that not only modified genes, but also the modification of regulatory interactions can play a paramount role in evolutionary innovation. This is evident from several genes that in all insects are involved in patterning the central nervous system but in *Drosophila* are additionally involved in the long germ segmentation cascade that must have arisen late in evolution. This cascade also involves two genes located within the HOX complex. They share characteristic sequences with the HOX genes but act much earlier and in different ways. Both may be considered HOX gene descendants put to novel regulatory functions. One of these genes is *bicoid* which so far has not been found outside the higher dipterans and thus may have acquired its basic patterning function late in phylogenesis. The other gene is *fushi tarazu*, involved in gene regulation at a peculiar level of long germ segmentation (defined by "pair-rule" expression); this gene seems to have evolved about ten times faster than the typical HOX genes (Akam et al, 1994) - an example of a "runaway" gene assuming new regulatory functions?

Finally, it was also changes in developmental gene regulation that have enabled *Drosophila* research to play a singular role in the discovery and analysis of the HOX complex. In both arthropods and vertebrates this complex directs the regional differentiation of the central nervous system, but it is only in insects that - owing to a divergence of regulation between the two phyla - the HOX complex directly controls the patterning of the body surface. Mutations in the latter function - leading for instance to four-winged flies - have triggered much of the molecular approach now revolutionizing our concepts of embryonic patterning mechanisms in insects and metazoans in general.

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* Note that individual genes occurring in several species are not identical as a rule, but are homologous, i.e. they share a high degree of sequence similarity and thus must have originated from the same ancestral gene.

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INTERNATIONAL COLLABORATION AMONG NATIONAL ENTOMOLOGICAL SOCIETIES

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Internationalism and isolationism tend to alternate over time. Most recently there have been disturbing signs of overzealous nationalism which when taken to extremes can cause serious problems. In spite of some recent examples of strong national, tribal, ethnic, and religious discord around the world, I believe that the trend toward international cooperation will prevail. Interdependence continues to increase among people of the world and likely will accelerate as the "information age" expands. The Internet allows low-priced instant electronic communication; its popularity, the increase of multinational business ventures, and the gradual recognition that people of all regions share more similarities than differences makes national isolation an anachronism. The revolution in electronic communication is only beginning to be felt and the boundaries of distance are being eliminated. Information is being democratized. We biologists, of course, have long realized that the natural world is oblivious to political boundaries. Global interconnections of environmental and other issues demand that we biologists play a leading role in educating and convincing people, and thereby politicians, that all of us truly are passengers on spaceship planet earth. We must work together to achieve sustainable development and improvement of the quality of life for all people.

Insects, ticks, and mites are the most numerous animals in species diversity and numbers and have tremendous direct and indirect economic importance. Because of this, entomologists may have a greater opportunity than other biologists to convince government officials and leaders in industry to support international cooperative projects.

Current status of International Cooperation Among Entomologists - International collaboration in science has increased during the past century (Storer, 1970; Luukkonen et al, 1992). Historically, such collaboration may have been due to increasing professionalization of science (Beaver and Rosen, 1978, 1979) and might be related to the formation of scientific societies, meetings, and conferences (Resh and Yamamoto, 1996). A recent study of international collaboration among entomologists involved analysis of authorship of the most recent 100 papers in 115 specialized journals (Resh and Yamamoto, 1996). Multicountry addresses for authors (indicating international collaboration) were recorded in 9.6% of articles examined. Whereas, frequency and success of collaborations depend ultimately on friendships, shared interests, available resources, and other subjective values, it seems likely that conferences, congresses, and scientific societies might play a role in initiating interactions among entomologists. For example, there is no doubt in my mind that the XX International Congress of Entomology will provide an opportunity and stimulus for international collaborations and that productive projects will result.

The Council for International Congresses of Entomology is the organization that provides for the continuation of these con-

gresses every four years. The Council has served entomology well. The question I raise today is whether the global entomological community seeks an expanded role for the Council and/or formation of another organization devoted to goals other than the responsibility of the international congresses every four years? Such goals might involve coordination of activities such as exchange of entomological scholars among countries, exchange of entomological journals, reciprocal memberships in national entomological societies, exchange of newsletters among national societies, issuance of position statements (biodiversity, pesticides, etc.), and other activities. Such a Federation of National Entomological Societies might also assist and promote joint meetings between member Societies, help improve education as well as advise on issues of entomological importance, promote programs and educational opportunities for professional entomologists (students included), improve intercommunication between Societies such as development of a directory to include officers and committees of member Societies, promote networking among members and journal editors, ensure maximum distribution of information on job opportunities, educational opportunities, promote exchange of entomological specimens, ensure exchange of information on any fast-breaking developments between member Societies, to act where possible as one voice, and develop joint policy action statements on such topics as animal welfare, scientific ethics, etc.

On the other hand, perhaps the establishment of a Federation of National Entomological Societies is not desirable or feasible at this time. If not, individual entomological societies might be persuaded to increase their interest and actions supporting international entomological activities. The Entomological Society of America (ESA) has increased its international activities during the past several years. Of the approximate 8,500 members of the ESA, 938 are from outside the United States. The ESA has a standing committee devoted to international affairs and a special representative for international affairs. The Society also has an excellent Library Enhancement Program to aid entomological libraries and scientists in several regions of the world where economic conditions preclude subscriptions to five journals published by the ESA. The gratis journal program is sub-divided to Sub-Saharan Africa, Central Eurasia (including newly independent countries of the former Soviet Union), and Latin America. Most of the libraries do not receive all five journals, although some do, but receive the journals most important for their speciality. For example, a medical library would receive the *Journal of Medical Entomology* whereas libraries devoted to agriculture would receive the *Journal of Economic Entomology*, etc.

The ESA also recently hosted an exchange entomologist, Dr. Bella Striganova, for six weeks with partial support of a grant from the American Association for the Advancement of Science (AAAS). The purpose of the visit was to exchange information on how the science of entomology is organized in the USA. For

example, how the ESA, universities, industry, and government interact to provide an infrastructure for the science. The ESA and many individual entomologists acted as Dr. Striganova's guides and teachers through this process. Hopefully, the information gained from the experience is being transferred to Russia and will be of help.

Further evidence of ESA's commitment to international cooperation among entomologists is the existence of the International Affairs Committee. That committee recommended and the Governing Board endorsed a change in the Society's Constitution to reflect a more global mission and scope. The modification heightens the commitment and responsibility of the ESA to the science of entomology that has no national or political boundaries. Additionally, the ESA Program Committee responsible for the programs at the annual meetings almost always extends invitations to foreign colleagues to present lectures at the meetings. Frequently the expenses of the lecturer are paid by the ESA.

Some of our more conservative colleagues might argue that international collaboration among Societies is a worthy goal, but this is the wrong time to initiate such efforts given the financial climate of most nations and the nationalistic attitude of some politicians. They would remind us that any programs, such as

exchange of scientists, cooperative research efforts, exchanges of publications, etc., would require central coordination through some organization and would need financial resources. In today's world of budget reductions it might be extremely difficult to generate financial support. I would argue that much can be done under existing conditions if there are sincere commitments by a few entomologists and a desire for greater international collaboration by a larger number of our colleagues. Even if a formal Federation of National Entomological Societies is not established much can be accomplished within the organizations of existing national Societies if the will to do so is present.

In conclusion, there is need for leadership to act as a catalyst in facilitating programs that encourage international collaboration among entomologists, although we all understand that ultimately success depends on individual initiatives. I have restricted my lecture today to allow time for a short meeting of those who may be interested in discussing international collaboration among entomologists and what steps should be taken.

Acknowledgements - Gratitude is expressed to Harry Bradley, Kaye Meckley, and Ray Everngam of the Entomological Society of America headquarters staff for providing information about the ESA's international activities.

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ENTOMOLOGY INTO THE THIRD MILLENNIUM

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Science is a product of the societies that support it. However much we might like to think of science as an independent international system of idea generation and testing, the fact remains that it is developed by and paid for by the society in which we happen to live and to which we and our families subscribe. Not all societies have shared, nor will share in the future, our views on evolutionary biology, natural history, or the freedom to ask questions about natural processes. The future of entomology is thus as much in our hands as members of society as it is in our hands as scientists.

The group photograph of the First International Congress of Entomology, taken in Bruxelles in 1908, epitomises the confidence and cohesion of our predecessors at the start of the 20th century. Broadly speaking, these participants belonged to one race and one social group - relatively wealthy, middle-class, and European. They knew each other; they shared each other's motivation and mores to a greater extent than our current entomological colleagues. But at what cost? The vast majority of mankind was excluded from this social group, and any knowledge acquired by mankind from outside the intellectual heritage of Greece and Rome was similarly excluded. To a large extent, entomology was a rich white-man's game. Any knowledge about the natural world, even pest control, generated by cultures in Australia or Africa, China or India, remained uncommunicated, sometimes despite written literature extending back for 3,000 years. Our science at the beginning of the century thus reflected most of the European social divisions then current; most of the women in the photograph are spouses, despite individuals such as Eleanor Ormerod, one of the first great economic entomologists, having made formidable scientific contributions.

Consider the changes this century. At one of our International Congresses participants will come from 70 or more countries, representing most of the ethnic and language groups to which our species has given rise, and entomological congresses in China or Brasil attract several thousand local participants. Entomologists now come from the full range of society, from peasants to plutocrats, and our president and vice-president are both women. But increasing numbers and diversity of individuals and sub-disciplines poses two fundamental problems for the future, the funding of an ever expanding array of knowledge, and then communication between this group of people as it continues to expand both numerically and socially.

Consider again that group photograph of 1908. The participants exude confidence in themselves and their society. In the words of the period, 'the world was their oyster'. The natural world had always been there; it was available to be explored, and provided a limitless source to be exploited. Not recognising the destruction already effected by mankind's territorial and population expansion, such as the extermination of larger animals across the Pacific islands or the deforestation of parts of China, they could not foresee the damage that would follow human popu-

lation increases resulting from pest and disease control. Malthus was for academics, not for the investment class to which most entomologists belonged. Our generation recognises the problems, as well as our professional responsibilities for ameliorating the conditions of life for future generations, but it remains to be seen if we have the political courage to do anything.

This then is the starting position for entomology into the third millennium: increasing numbers of entomologists and a burgeoning literature in many languages and sub-disciplines, with resultant problems in communication; increasing diversity and expense in the techniques employed to study insects, with resultant problems for funding the increased costs of all but the most vital of activities; decreasing diversity of insects as natural habitats are destroyed, with resultant general impoverishment of the human environment as well as increasing constraints on our range of insect research options; and an increasing human population with all the social and educational problems that implies. This is surely the reality? Business will not be 'as usual' into the next century, although we cannot be sure what business will actually be like. We cannot predict the future, but we can certainly plan and attempt to create that future - instead of behaving like the images in the 1908 photograph, confident that the world will continue to supply their needs.

We cannot be sure where entomological research will lead, the investigation of the structure and functioning of insects as well as their relationships to each other and the rest of the world. Such investigations depend on serendipity, and the fertility of the minds that are involved in insect science. But we can look at some of the problems outlined above and consider our possible reactions. In considering the future, it is no co-incidence that the three Symposia and Workshop organised in Section 24 of this International Congress were each about human problems rather than science in a narrow sense - education, communication and quarantine. Because if we fail in these areas, then we will further limit the resources available to society for studies on insects.

Scientific research has always been expensive. The mathematicians and astronomers of ancient Egypt or India must have cost their wealthy patrons dearly. Wealthy patronage, often largely for reasons of self-interest, continued to be the funding source into this century, until it was superseded by state funding on the grounds of generalised public good. But the costs of science continue to rise as research workers discover new methods that open up new horizons for exploration. And the level of funding available for science from the public purse cannot be expected to keep pace with this rise in costs - nor yet with the rise in research possibilities. The funding base must inevitably level-off, leaving governments as well as each of us with ever more difficult decisions on priorities.

In most countries, the state has now reverted to the self-interested views of many wealthy patrons; government accountants

expect to see an early financial return on any investment of public money in scientific research. Henry Ford is reputed to have said that history is bunk, but at times our politicians and their accountants give the impression that they consider that the future is bunk, or at least has no existence beyond the period of our current grant. This places constraints on planning biological studies, because these often need a long lead-time. As a result we need to develop more dynamic ways of planning research such that specific short term results are obtained that are useful to our fundees, whilst we progress toward the more general objectives that we ourselves consider to be important. Regrettably, scientists rarely make good politicians.

The diversification of science certainly involves serious financial problems. For example, as a museum taxonomist my only expensive research tool (apart from the museum collections themselves) for 30 years was one high-quality, phase-contrast microscope. However, my capital and recurrent expenditure rose sharply in recent years in line with expanded research possibilities - computers of ever increasing sophistication, equipment for rearing and observing behaviour, facilities for field research, and participation in DNA methods for distinguishing taxa. This expansion in research costs inevitably results in fewer people being employed. Similarly, entomological libraries in Britain find that the cost of journal subscriptions has been rising at nearly four times the rate of inflation; the cost of binding and accommodating books has similarly increased, and this at a time when the number of publications on insects is rising rapidly. As a result, many librarians must annually cull their journal and book purchase lists in order to reflect only the work currently in progress in their institutes; it is not possible for any institute to maintain a complete collection of all books and journals referring to insects. The CSIRO library in Canberra houses almost 500 serials concerned in some measure with insects, but China alone now publishes annually the equivalent of half of that number of periodicals containing information about insects.

The application of 'market forces' to the funding of research has unforeseen problems, because the entomological 'market' itself is not stationary. At one time the market was clearly discipline-orientated, with most universities and agricultural research organisations having an 'entomology department'. Now, however many departments have a different orientation - the life sciences, or plant protection for example. Many people working on insects in fields such as plant production, public hygiene, conservation, or water quality, do not automatically regard themselves as 'entomologists'. As a result, the market for books is changing as the traditional discipline-orientation of these potential purchasers changes. One major publisher of review journals has reported annual sales reductions over the past few years of 2% per year, with profit losses nearer 5% per year. An accountant can readily calculate that each paper published in such a commercial journal must generate more than 5,000 US\$ in gross sales. The market for entomological information is thus shifting, and individual costs are escalating; this has downstream effects both on how we plan to distribute information in the future, and how opportunities for research are perceived.

The next generation of students needs to be attracted to insects, as objects of interest, beauty and importance to their lives. Didactic methods of teaching are no longer appropriate in this computer and television driven age. We need methods that carry greater impact in communicating to students our knowledge, excitement and understanding of the natural world, using good images in books and on computers. This is now happening for younger children. Why then do our teaching methods for older students remain so prosaic? Students are expected to learn

considerable detail about insect structure - all from books. In medicine, it is already possible to obtain on cd-rom complete dissections of the human body obtained from serial sections. In the future, student surgeons will be able to practise dissections on such a 'virtual-body' - a computer image. Why is there no cd-rom compendium of insect structure available, so that students could search at their computer terminal for the images and information they need - on mandibular structure or wing venation. Increasingly students prefer to use terminals rather than libraries, and we need to plan our teaching accordingly. By imparting basic facts more efficiently than in the time-honoured system of lecturing and taking notes, we could leave more time for teaching - that valuable practice of stimulating interest and thought in others.

Virtual imaging must have a significant future in entomology. Already it is possible to 'grow' on screen a virtual cotton plant, and to vary growth parameters. This technique can be used, for example, to investigate the possibility of improved plant architecture in order to improve the interception of light by leaves, and thus create a specific objective for plant breeders. But the next stage in virtual imaging will include the effect on plant growth of insect pests, and subsequently the effect of parasites on pests. The possibility of carrying out virtual insecticide trials on screen, varying the droplet sizes, wind speeds, concentrations and frequency, with only the most effective treatments needing to go through the expensive process of 'ground-truthing', holds out the prospect of reduced costs, shorter experimental periods and lower usage of chemicals.

As a small science, entomology is scarcely keeping pace with the changes in communication. Many thousands of scientific journals are now available via the Internet, with copies available by fax on request. More than 700 scientific journals worldwide are now published on-line, and the number is increasing rapidly. At least one such journal, in biochemistry and medicine, has the facility of reproducing on screen abstracts of all of the papers quoted in the reference lists of each of the papers it publishes - a complex system of hyperlinks that is currently too expensive for entomology. But entomology, and particularly taxonomic entomology, is often concerned more with publication in terms of priority rather than in terms of information dissemination. Libraries are increasingly expensive to build and maintain; the future for information dissemination thus seems to lie in electronic methods, although the problems of priority and archiving such material have yet to be solved.

The new check-list of Lepidoptera from Australia, a vast archival source involving over 25,000 names, will be published on cd-rom by CSIRO this year. But large quantities of information about insects, particularly that which is required by only a few workers worldwide in any one year, could be communicated to the users much more cost-effectively using this technology than as books. Electronic information and identification systems are being produced in entomology that will go far to assist global communication about insects. Bear in mind the many thousands of periodicals and books in which information about insects is now published - and few of the hundreds published in Asian countries are available in Africa or South America. Increasingly we all turn now to our terminals, rather than to our libraries, to find out what is going on in our own fields as well as in surrounding fields of biology.

Communication between the many sub-disciplines of insect biology becomes ever more difficult as the complexity of our studies expand. It is doubtful if the traditional all-embracing approach to entomological training remains valid, particularly if the students are themselves intended to contribute professionally

at one or more of the current cutting edges of insect science. Increasingly, students at doctorate level are unable to recognise members of common beetle or fly families. But is this such a great loss? The holistic ideal is our aim, because ultimately we try to understand how an individual and its species functions, how it contributes to ecosystems, and how the interactions between species and ecosystems have evolved and facilitated evolution. We examine the present in detail, to understand the past and predict the future. But there are ways of achieving such holistic ends without expecting every student to master the whole range of insect science. Increasingly, research grants given for collaborative and inter-disciplinary work bring together our sub-disciplines, with mutual enlightenment and stimulation. Understanding the diversity, functioning and evolution of systems through collaborative work is likely to be of greater benefit to mankind than concentration on particular taxa, even one as all pervasive as the Insecta.

Meanwhile the world around us will presumably not become any less complex. The human population is likely to continue to rise, the natural world is likely to continue to become more

depauperate, and competition between nations for limited global resources is likely to become sharper. The global marketplace has led to a global pest and disease spectrum. Already, all of the 40 major weed species of the world are found in Australia, as are 20 of the 35 most important thrips pests. A similar picture could be found in many other countries. This places strains on our quarantine services in the ever unpopular problems of controlling the international movement of people and their produce. Our increasing mobility places at risk not just our crops but also our natural resources. Perhaps one major growth area for entomology will be in association with the expanding tourist and leisure industries. We all need the benefits of natural environments to recover from the stresses of industrialised living. Through enhanced conservation programmes, involving improved education and communication about biological diversity, both its commercial and intellectual value, perhaps we can maintain our research budgets, contribute usefully to society, and continue with that human activity we all find most pleasurable and stimulating - the study of insect life.



Figure 1. - Participants to the 1st International Congress of Entomology, Bruxelles, 1908.

ABSTRACTS

Section 1
Systematics and
Phylogeny

01-002

A "TOTAL EVIDENCE" CLADOGRAM FOR THE
ARTHROPODA
W. Wheeler

ABSTRACT NOT RECEIVED

01-001

THE ARTICULATES: THE METAMERE AND TAGMA CONCEPTS
IN THE LIGHT OF MODERN DEVELOPMENTAL MORPHOLOGY
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Processes governing the parasegmentation, segmentation, and subsequent tagmatization, well known in *Drosophila*, are compared with the fragmentary knowledge of these phenomena in other arthropods, onychophorans, and annelids. All arthropod segments are probably homologous among all arthropods; the concurrence of both the parasegments and segments is probably probably characteristic of all the arthropods and annelids. The annelid segments (and segments of the hypothetical soft-bodied arthropod ancestor) cannot be identified with parasegments. The alleged Snodgrassian "primary segments" (antecosta-antecosta) are identical with no identified developmental body metameres in arthropods. Evolutionary morphology as well as embryology of crustaceans and hexapods (cell-lineages, expression of segmentation and homeotic genes, mitotic domains) suggest that the basic boundary subdividing the mandibulate body runs within the mandibular segment, between protocephalon (pregnathal region) and protocorm (trunk incl. maxillary segments). The "typical" mandibulate head is not developed in all the Mandibulata (Crustacea + Hexapoda + Myriapoda) and has evolved several times in parallel. Postmaxillary trunk tagmosis of anostracans may be homologous to that of insects (10-11 pregenital trunk segments with Antp, Ubx, and abdA genes coexpression, 2 genital segments with AbdB gene expression).

01-003

THE GROUND PLAN AND BASAL DIVERSIFICATION OF
HEXAPODS
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The tagmosis pattern is the most obvious groundplan autapomorphy of the Hexapoda as usually delimited, but evidently an ad hoc explanation is, then, required to account for the small number of abdominal segments in the Collembola. The problems inherent in a detailed reconstruction of the hexapod groundplan are inextricably linked with the problems concerning the status and affinities of the conventionally recognized taxon 'Diplura'. Do the 'campodeoid' and 'japygoid' lineages really constitute a monophylum? If so, are its closest relations with the Entognatha s.str. (Collembola+Protura) or are they with the Insecta s.str. (Archaeognatha+Dicondylia), rendering entognathy homoplasious? If not, do all 'entognathan' lineages constitute a 'Hennigian comb', or are the 'japygoids' the sister group of the Insecta s.str. as has recently been suggested? While the monophyly of the Dicondylia seems to gain widespread acceptance, the question about the sister group of the winged-insect clade remains open. Are the families currently grouped together in the 'Zygentoma' really a monophylum, or are the Lepismatidae closer to the Pterygota than the Lepidothricidae which allegedly have retained a 'ligamentous' cephalic endoskeleton? And where do the Nicoletiidae fit in? The basal split within extant Pterygota arguably remains unsatisfactorily resolved. An in-depth examination of the Ephemeroptera/Odenata/Neoptera trichotomy is called for. Emphasis should be placed on non-alar characters which lend themselves to polarization by the outgroup criterion.

01-004

HOW TO FIND AND TEST RELIABLE HIGHER LEVEL CHARACTERS IN INSECTS

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The higher systematics of Insecta is presently experiencing confused times. Much energy goes into sophisticated **processing** of quite uncertain characters, but very little into **primary research** on characters, the morphological backbone of phylogeny. Parsimony (aiming not for a grade of A, or F, but for C-) works well enough for specics, but is not good enough at higher levels. Higher level apomorphies are nodal transformations which occurred from ca 600-200 m.y. ago. They were few and are now often obscured in modern insects. They are mostly interlocked in old major structures, such as legs, wings and mouthparts, and reachable by two approaches: 1. Panarthropod comparative anatomy, and 2. Developmental genetics. Both painstakingly gather evidence for the vanished, nodal ancestor of a taxon, known as a groundplan, from which all members can be derived. Used by Snodgrass and Hennig, the groundplan is the best way towards understanding major structures and obtaining reliable rooting for directional morphological transformations. It reveals long series of character changes in clades of orders, suborders and superfamilies. Recently, cross-testing with genetics has already confirmed the proposed basal anatomy of the leg (polyamy, mandible, leglet, proleg, mouthparts and the wing identity: JKP, *The Insects of Australia* 1991). Present progress in pterygote phylogeny is in the improved homologisation and in new groundplans of the higher taxa based on wing venation and wing articulation. A new, improved phylogenetic tree of pterygote orders is offered.

01-006

THE MANDIBULAR ARTICULATION OF MAYFLIES (INSECTA: EPHEMEROPTERA) AND ITS IMPLICATIONS FOR THE PHYLOGENY OF THE BASIC PTERYGOTE LINEAGESA.H. Staniczek

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To unravel the groundplan condition regarding the mandibular articulation of Ephemeroptera several basal mayfly nymphs have been investigated by manual preparation as well as microscopic sectioning. The results indicate the presence of three points of mandibular attachment to the cranium in the groundplan of Ephemeroptera. The anterior point of attachment is represented by a lateral mandibular groove that fits into the inner margin of the inflected cranium. This structure is interpreted as an autapomorphy of the Ephemeroptera. The middle and posterior points of articulation are homologized with the anterior (secondary) and posterior (primary) mandibular joint of other Dicondylia.

The development of an additional mandibular attachment in Ephemeroptera is interrelated with a different position of the anterior tentorial arms. The anterior tentorial pit in mayflies has a more posterior position compared to the condition in other Dicondylia. The cranial condylus of the secondary mandibular joint in mayflies is located close to the anterior tentorial pit. The backward shift of both anterior tentorial arm and cranial condylus is regarded as an autapomorphic character of the Ephemeroptera, too.

Besides these derived characters the mandible of Ephemeroptera retains the groundplan condition of Dicondylia in several aspects: The secondary mandibular joint is not visible externally, and its cranial condyle is not formed by the clypeus. The primary mandibular articulation is located dorsally of the secondary one, which brings about an oblique axis of mandibular movement. This resembles a plesiomorphic arrangement as it is present in *Zygantoma*, too. In contrast all other Dicondylia have developed an almost horizontal axis of mandibular movement. This character distribution could count for a sistergroup relationship between Ephemeroptera and Metapterygota.

01-005

LIMB BASE MORPHOLOGY OF THE ARTHROPODA: A REVIEW OF COMPETING MODELSG. CASSIS, G.D. EDGEcombe, G.D.F. WILSON AND M.R. GRAY
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Recent challenges to the monophyly of the Mandibulata and Tracheata stem from both molecular and morphological data. The theory that insects are derived from a paraphyletic Crustacea has a background in the comparison of derived crustacean limbs (Eumalacostraca) with those of insects. This theory presumes that the Crustacea lack defining synapomorphies. Numerous shared derived characters of Crustacea refute the notion of Crustacea as a grade ancestral to insects or any other arthropod taxon. Homologies of limb base segments across the arthropods have been only partially resolved. Walossek has proposed that the "coxa" of the Crustacea is a new structure, not present in basal arthropods, and not present in the most recent common ancestor of the Mandibulata. Fossil stem group crustaceans have proximal elements of the legs, particularly the "basis", that can be homologised with the stem group chelicerates (e.g. trilobites). The precise identity of the basis in the Tracheata is unresolved. However, the subcoxal and coxal leg elements of the Tracheata are derived characters of that group. The primitive absence of the subcoxa in crustaceans conflicts with the Kukalova-Peck model for leg base homologies throughout the Mandibulata and more broadly Arthropoda. This is supported by the absence of a pleuron in the Crustacea. Furthermore, problems with the polyramous model for basal arthropod appendages are that crustaceans and early chelicerates have a fixed and conservative relationship between endopod, exopod and basis, and lack multiple exites. Therefore, the presence of a stylus or multiple styli is a weak criterion for establishing polyramy at the base of the Arthropoda.

01-007

A "TOTAL EVIDENCE" CLADOGRAM FOR THE HEXAPODA

J. Carpenter (Tifton-United States)

ABSTRACT NOT RECEIVED

01-008

SPECIES NUMBERS OF HEXAPOD GROUPS IN A PHYLOGENETIC CONTEXT
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Data on the number of described species currently recognised as valid for major hexapod groups are reviewed, the evidence with respect to actual numbers of extant species examined, and methods for estimating undescribed species numbers critically evaluated.

Hexapod "success" measured in species-richness terms is manifested mainly through a rather small number of endopterygote clades. In discussing this, the following are among the questions addressed: What are the principal correlates of such success in terms of lifeways, aptations and resources utilised? Are recent accounts of how species-richness of the major groups may have varied through geological time reliable? Have some clades peaked already and, if so, why?

01-010

MAPPING OVARIOLE STRUCTURE ON THE HEXAPOD CLADOGRAM
J. Buning (Erlangen-Germany)

ABSTRACT NOT RECEIVED

01-009

MAPPING MODES OF EMBRYOGENESIS ON THE HEXAPOD CLADOGRAM
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Comparative embryology of Hexapoda has revealed several characters which appear phylogenetically informative. The distribution of such characters (including among others the cleavage, embryonic membranes, amnioserosal fold, blastokinesis, germ type and germ layer formation) upon a 'total evidence' cladogram will be examined. The Protura are excluded from consideration, since their embryology still remains unknown.

1. The monophyly of the taxon Ectognatha (= Insecta *s.str.*) as well as of the Dicondylia seems to be supported by important embryological apomorphies.
2. Members of the Entognatha share many embryological plesiomorphies, but the common mode of entognathy formation arguably suffices to suggest their monophyly (*i.e.*, it is a synapomorphy of Collembola and Diplura). The Diplura are also recognized as monophyletic on the basis of detailed similarities in entognathy formation in the Rhabdura and Dicellurata, as well as their sharing of an abdomen with only ten segments.
3. Available data from comparative embryology of the Pterygota do not permit far-reaching conclusions on order-level phylogeny. Some comments on pertinent topical problems will be given.
4. Comparative embryology so far supports a hexapod phylogeny formulated as: Entognatha (= Collembola + Diplura) + Ectognatha [= Monocondylia (= Archaeognatha) + Dicondylia (= Zygentoma + Pterygota)].

01-011

MAPPING EYE STRUCTURE ON THE HEXAPOD CLADOGRAM
H.F. Paulus (Wien-Austria)

ABSTRACT NOT RECEIVED

01-012

MAPPING CIRCULATORY SYSTEMS ON THE HEXAPOD CLADOGRAM

G. Pass (Wien-Austria)

ABSTRACT NOT RECEIVED

01-014

A MOLECULAR PHYLOGENY OF THE FAMILIES AND ORDERS OF COLLEMBOLA (ARTHROPODA: HEXAPODA) USING THE 18S r-RNA GENE

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We investigated the phylogenetic relationships of the Families and Orders of Collembola, with special reference to six groups that traditionally have been problematic, or that have become problematic with the implementation of a cladistic philosophy to the taxonomy of the group. Some authors have questioned the monophyly of the order Poduromorpha, and the supraordinal categories Arthropleona and Symphypleona s.l. In addition, the specific phylogenetic affinities of Poduridae, Actaletidae, and Tomoceridae have not been resolved. To clarify these relationships we obtained complete sequences for the 18S rRNA gene from 21 species representing the families Hypogastruridae, Onychiuridae, Poduridae, Neanuridae, Isotomidae, Actaletidae, Tomoceridae, Entomobryidae, Neelidae, and Sminthuridae s.l., and all four orders. We also sequenced the 18S gene from a jumping bristletail (*Microcoryphia*), and a house centipede (*Chilopoda*), which together with sequences from *Artemia salina* (Crustacea), and *Tenebrio molitor* (Coleoptera) (obtained from Genbank) were used as outgroups in the phylogenetic analyses. Phylogenetic analyses were performed using parsimony. The monophyly of Poduromorpha was supported by all analyses. Support for a monophyletic Arthropleona was ambiguous and cannot be resolved at this time. A monophyletic Symphypleona s.l. was never supported. The placement of Poduridae in Poduromorpha was always supported, but its affinities within the order could not be unambiguously resolved. Most of the iconoclastic propositions of the phylogenetic affinities of Actaletidae were rejected, but the question remains of whether this family is the sister group of Isotomidae or basal to all other Entomobryomorpha. The proper placement of Tomoceridae could not be resolved because in most analyses this taxon clustered with different groups. We attribute the failure of 18S to support the monophyly of groups well supported by morphological characters to differences in rates of evolution among lineages, and to low stemminess in the most parsimonious trees, presumably resulting from rapid radiation in relatively short time spans.

01-013

MAPPING AQUATIC LIFE-STYLES ON THE HEXAPOD CLADOGRAM

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Insects show at least 12 types of life histories in which one or more stages are aquatic. Morphological studies suggest that these aquatic life-styles have evolved several times from completely terrestrial ancestors. In this presentation I map the distribution of aquatic life-styles on the latest 'total evidence' cladogram, and discuss congruences, conflicts, and insights that arise.

01-015

Evolutionary pathways of larval eyes in Coleoptera

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Coleopteran stemmata are derived from modified imaginal ommatidia. The plesiomorphic state are probably 7 stemmata (only Dytiscini) as in some other holometabolan orders. But in most families the basic number is 6. An ultrastructural investigation of many coleopteran taxa revealed that the plesiomorphic stemma is a modified ommatidium with an acone crystalline cone, consisting of 8 retinula cells arranged in two levels; the two primary pigment cells are lacking or modified in two undifferentiated comeagenous cells. Such a basic larval ommatidium can be found in Staphylinidae (*Lesteva*) and Lagriidae. In most cases a crystalline cone is lacking or replaced by an epidermal layer of hyaline cells. Further modifications are (1) fusing of single stemmata to double-stemmata or fusion of higher numbers of stemmata with a respective multiplication of cell numbers or (2) multiplication of cells in each stemma without fusion. Diminution of stemmata number can be a consequence of (1) reduction or (2) fusion. Many Staphylinidae, Scirtidae, Pyrochroidae, Cerambycidae and others belong to case 1, some other Staphylinidae (*Atheta*), Elateridae, Cantharidae, and some Tenebrionidae represent case 2. In most Adephaga we find 6 stemmata with higher cell numbers originated by secondary multiplication. A special case is the doubling of cells in all stemmata without reduction of the lens number. So far as I know all Byrrhoidea, Dryopoidea, Elateroidea and Cantharoidea have as a basic situation of 6 double-stemmata each with 16 retinula cells. Starting from this situation the single lens in Elateroidea and Cantharoidea is a fusion of the six double-stemmata. In *Cantharis* we find two groups of retinula cells each consisting of 48 cells (= 3 X 16), in *Drilus* three groups each with 32 cells (= 2 X 16). In all cases we have a total number of 6 X 16 retinula cells. From these differences in cell numbers and cell arrangement I conclude that the single lens eyes in Coleoptera larvae are the result of independent evolutionary pathways.

01-016

PREVENTIVE PEST MANAGEMENT: MANIPULATING EVOLUTIONARY STRATEGIES OF PEST SPECIES

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The basic paradigm and strategies of contemporary pest management have not changed very much for the last several decades, although the level of pesticide application is moderated. We continue to struggle with pest resistance against pesticides and environmental degradation by agrochemicals. Meanwhile, however, our world has changed dramatically. The planet is now inhabited by 5.75 billion humans with an addition of 100 million per year and the global environment is increasingly at risk because of increasing human activities. In this rapidly changing world we cannot afford to continue this management practice indefinitely.

Pest management should be anticipatory, predictive and preventive as well as managing pest populations. It should take on ecosystem management strategies focusing on the biology and ecology of pests and the biological impact of pests on host plants with the least environmental costs. Pest species evolves in response to the changing conditions of their genetic, ecological and habitat environments, as it continually interacts with other ecological cohorts including host plants. Pest management strategy should focus on manipulating the evolutionary process of pest species to reduce the impacts of pest activities to agricultural production and forest management rather than the population density in the context of ecosystem management. This strategy requires a multi-level approach encompassing the issues of intraspecific variation, demography, community dynamics, and ecosystem process. In this paper the issues involved in intraspecific variation among and within populations of pest species will be discussed with respect to pest management and a new approach to integrated pest management will be explored.

01-018

CONTRIBUTIONS AND APPLICATIONS OF MOLECULAR TECHNIQUES IN PEST MANAGEMENT

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A variety of molecular techniques to study systematics, evolution and population genetics of insects have become available to entomologists during the past two decades. In conjunction, the breadth of applications has also increased to include basic and applied entomology. Of particular interest is the potential use of the emerging molecular techniques to study various aspects of pest insects and natural enemies and apply the information to enhance existing integrated pest management programs. In this presentation, various molecular techniques and their applications will be reviewed with relevant examples from pest insects and natural enemies. The emphasis will be on (a) use of population-level markers to study gene flow in pest insects and natural enemies, (b) use of DNA sequence analysis to infer phylogenies of groups that contain one or more pest insects, which in turn can provide insights into evolutionary pathways of specific traits (e.g., adaptation to crop plants, host range), and (c) use of DNA sequence analysis to infer phylogenies of natural enemies and study the evolution of host range and other behaviors.

01-017

SPECIES CONCEPTS IN PEST MANAGEMENT - THEORY AND PRACTICE

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The importance of biodiversity in sustainable agriculture is widely emphasised and species are generally used as the units of such diversity. The correct identity and recognition of species of insect pests and their predators and parasites is one of the most critical contributions of systematics to scientific pest management.

As Ernst Mayr (1983) has emphasised, the species concept in biology has consistently remained controversial. Traditionally insect species have been defined in terms of clear morphological differentiation. In practice most species of insects are still described on a basis of dead preserved material and remain *morphospecies*.

Ideas of species as reproductively isolated entities in the field led to the development of the many variants of the *biological species* concept. An important consequence of the biological species is the recognition of reproductively isolated cryptic or sibling species that show no clear morphological differentiation but which are reproductively isolated. In practice species are characterised by markers that may be morphological, cytological, behavioural, molecular, *etc.*, but which indicate reproductive isolation.

Difficulties with 1. asexual and obligately parthenogenetic forms, and 2. populations isolated in space (allopatric), together with a desire to apply cladistic techniques have led to the rejection of the biological species in favour of a *phylogenetic species* concept by many systematists. Problems for pest management of such a change will be considered. They include particularly the possibility of ignoring sibling species. Examples from groups of tropical insect pests and their parasitoids will be discussed.

01-019

SYSTEMATICS RESEARCH IN SUPPORT OF BIOLOGICAL CONTROL

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Systematics provides critical support for biological control by providing baseline information. In this paper we summarize how systematics information impacts biological control with emphasis on how advances in methods, theory, and information transfer provide the opportunity for enhanced interaction between systematists and biological control workers. We also document how inadequate systematics adversely impacts control programs.

Most insect groups are poorly known, and information concerning species must often be predicted from their closest relatives. Phylogenetic systematics, by providing testable hypotheses of relationship within groups, gives workers a framework of relationships with which to make these predictions about native home host range, natural enemies, environmental tolerance, biology, behavior, etc.

Molecular systematics studies can complement more traditional methods and have allowed the solution of species and subspecies level problems of importance to biological control programs, particularly the discrimination of sibling species, geographical races and biotypes.

Advances in informatics (distribution of information via electronic means like the World Wide Web) promises to make the data from systematic studies more readily available to a wider audience and make it easier for non-taxonomists to make informed decisions about taxonomic problems.

Despite many recent advances in methodology and technology, great gaps still exist in our knowledge of pivotal groups used widely in biological control. Many areas, even in relatively well collected regions such as Europe and the United States still contain many undescribed taxa of potential biocontrol importance. In addition, there are numerous groups which are heavily utilized in biocontrol, but for which modern comprehensive systematics studies are still lacking. We see no easy solution to this problem beyond increasing funding for basic systematics research and closer coordination between systematists and biocontrol workers.

01-020

MOLECULAR PHYLOGENIES AND THEIR APPLICATION TO BIOLOGICAL CONTROL

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For the use of parasitoids or predators in biological control, molecular systematics has or will have impact on two important areas: 1) the identification of species or populations, and 2) the characterization of monophyletic lineages, whose members could share similar biological attributes. Chalcidoidea is likely the most important group of parasitic wasps for biological control programs; however, little progress is being made toward understanding the relationships of the included organisms using morphological techniques alone. Fundamental questions exist regarding the monophyly or relationship of most of the major assemblages such as the Aphelinidae, Eulophidae, Eupelmidae, Trichogrammatidae to name a few. Molecular techniques being used for the phylogenetic analysis of Chalcidoidea are reviewed, with an emphasis on understanding the monophyly and relationships of the Aphelinidae using nucleotide sequences of 18S, 28S D2 expansion and ITS2 regions of the rRNA transcript. Morphological features are compared with the resulting trees. Attributes of each of the genetic regions are discussed with regard to their application for the study of relationships within genera of Aphelinidae.

01-022

LYGUS BUGS: THE INTERFACE BETWEEN ANALYTICAL SYSTEMATICS AND PEST MANAGEMENT

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Species of *Lygus* (Heteroptera: Miridae) cause economic losses to a wide range of crops in North America and Europe. As a consequence of its impact on agroecosystems, *Lygus* continues to be the subject of much basic and applied research. Yet, until recently, there has not been a reliable model of the relationships among the species, nor have there been reliable methods to routinely identify the species; this is largely due to generally subtle differences among and a great deal of variability within taxonomic characters. We report on a broad systematic analysis of *Lygus*, using morphological and molecular data and morphometric and phylogenetic methods, focusing on the points of conflict and congruence observed between morphological and molecular approaches. Comprehensive systematic analyses are shown to provide a testable framework upon which life history strategies can be studied and biological control efforts and identification technologies can be developed. This approach has allowed for a focus on specific questions about the phylogenetic placement of the economically important species, their geographical distributions and potential host plant ranges; all are issues of importance in the selection of biological control agents, regulation against the introduction of new pest species and the development of new crops.

01-021

RICE INSECTS AND PEST MANAGEMENT:
IS TAXONOMY IMPORTANT?

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Rice (*Oryza sativa*) is by far the most important cereal crop grown in tropical countries. Over 800 insect species are known to damage rice while it is growing or in store. A considerable amount of information on rice pests, and their predators and parasitoids has been amassed. This has enabled increasingly sophisticated integrated management programmes to be developed, particularly as a result of reductions in agrochemical usage. To what extent have these advances in pest management been due to taxonomic knowledge? Can successful management of pests be achieved without taxonomic studies? This idea is examined with rice insects providing examples for discussion.

01-023

DISSEMINATING BIOSYSTEMATIC INFORMATION:
YESTERDAY, TODAY AND TOMORROW.

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From Linnaeus to today, the content of BioSystematic Information has not changed much. Likewise, the demands of users: they want to know what it is, what it does, and when and where it does it. What has changed is the magnitude of information, from a few thousand to millions of data points, and the media on which to disseminate it, from the printed page to a full range of electronic formats, such as CD-ROM (Compact Disk-Read Only Memory) disks and WWW (World-Wide-Web) on the Internet. This explosion of information and multimedia, at a time when support for Systematics is declining but awareness of the importance of BioSystematic information for the sustainable use of Biodiversity is increasing users' demands, provides the challenge for the new century. How the systematists in Washington are meeting the challenge is illustrated and demonstrated with products, from on-line databases to expert identification systems.

01-024

INSECT COLLECTIONS AND PEST MANAGEMENT
E. Nielson (Canberra-Australia)

ABSTRACT NOT RECEIVED

01-025

Plant Protection
The Biosystematic Impediment to Success
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Attention is drawn to the vital importance of sound biosystematics in plant protection programmes, where economic and environmentally friendly systems have become imperative to lower crop production costs and achieve sustainable agricultural development. The need for user-friendly aids for identification for plant protection personnel and quarantine services is emphasised whilst recognising the continuing and increasing need for up-stream biosystematic research and keys from which such aids can be developed. The role of BioNET-INTERNATIONAL in removing the present biosystematic impediment to improved plant protection and quarantine is described.

01-026

THE EVOLUTION OF REPRODUCTIVE AND SOCIAL BEHAVIOURS IN
COCKROACHES: A PHYLOGENETIC ANALYSIS
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Cockroaches present a diverse array of reproductive and social behaviours. Reproductive behaviours comprise oviparity, ovoviviparity and viviparity. Oviparous species are themselves diverse with respect to the time females carry their oothecae and the way they provide them with water. Social behaviours comprise presociality and subsociality. There are also many non-social species. Several hypotheses have been made concerning the evolution of these behaviours (e.g. Roth, 1989; Nalepa, 1988). These hypotheses are tested using cladistic phylogenies inferred with morpho-anatomical characters (Grandcolas, 1994, 1996). Comparing phylogenetic patterns with previous evolutionary hypotheses, several points can be raised concerning the evolution of reproductive and social behaviours. Previous hypotheses were biased by *a priori* evolutionary models. Subsociality was hypothesised ancestral to cockroaches because subsocial cockroaches were thought to present many relict behaviours of the common ancestor of the cockroaches and the social group of Termites (Nalepa, 1988). According to phylogeny, subsociality appeared convergently in several cockroaches and was not ancestral to all cockroaches. Features associated to subsociality, such as symbiosis with Protozoa and xylophagy, recently appeared in the same way (Grandcolas & Deleporte, 1996). Reproductive behaviour was supposed to have evolved from more simple systems toward more elaborated ones. The systems which seemed intermediary elaborated were thought to be intermediary evolutionary stages (Roth, 1989). According to phylogeny, reproductive behaviour evolved several times convergently toward ovoviviparity from ancestral oviparity, and once toward viviparity from ovoviviparity. Carrying oothecae and providing them with water in oviparous species is not an intermediary condition leading to ovoviviparity but has evolved independently (Grandcolas, 1996). Using this phylogenetic perspective, cockroaches appear very modern insects contrary to the current opinion which considered them archaic relicts from Palaeozoic period. They do not show many ancestral characters and they have evolved toward very complex reproductive and social behaviours.

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01-027

ECOGEOGRAPHICAL DISTRIBUTION OF ORTHOPTERA
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The spatial approach (including the regional one) allows us to evaluate biogeographical regions for the Palaearctic Orthoptera. Each of them is determined by both geographically and ecologically. From the biological point of view every region is an aggregate of species populations, united by a territory. It may be described as an historical combination of the endemic and widely distributed Orthopteroid species, and also as a specifically arranged aggregate of populations, regularly distributed over local landscapes. These populations form corresponding communities in habitats. The another essential problem is to estimate permeability of different barriers on the various scales. So we can determine the possible and optimal ways for species spreading (migrations, wanderings), colonizing new landscapes and regions, and creating new populations. In this way, we may to understand the problems of a biological diversity of Orthoptera more correctly, because we can evaluate both a taxonomic diversity and diversities of populations and communities too.

01-028

BIOGEOGRAPHY OF THE PALAEARCTIC PAMPHAGIDAE (ORTHOPTERA)

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The Pamphagidae are spread over the whole Africa, the Mediterranean area and the Palearctic Asia. Of the subfamilies, the Eliotropinae are all restricted to the southern Africa and the Porthetinae are essentially living in the southern Africa with 14 genera. The Akicerinae have a markedly disjoined distribution: they are present in the southern Africa (5 endemic genera) and are also spread over western and central Asia with 18 genera and in the Mediterranean lands with 5 genera. The subfamily Pamphaginae is wholly Palearctic. The Pamphagidae most likely differentiated from a Gondwanian stock of Pamphagoidea and originated in the southern Africa in Mid-Cretaceous when the African plate was already separated from the S. American, Indian, Malaysian and Antarctic-Australian plates. The significant presence of Pamphagidae in the Palearctic Region (more than 350 species versus less than 170 Afrotropical species) can be ascribed to two biogeographical events which took place in the Palaeogene when some plate fragments, the terranes, broke off the northern edge of the African plate and, crossing the Tethys, rafted onto the southern edge of Eurasia, carrying there some stocks of Akicerinae and Pamphaginae. A western terrane (Alborana) accreted with the Iberian-Sardinian-Corsican plate, which afterwards broke up and gave rise by vicariance to the 14 genera of Pamphaginae (the whole tribe of Eurypteryphini included) that populated Spain, Sardinia, Sicily and the Maghreb. One or more eastern terranes accreted with the Afghanian-Iranian edge of the Eurasian plate, allowing the evolutionary explosion in Asia of further 21 genera of Pamphaginae and 18 genera of Akicerinae. Another evolutionary explosion of the Akicerinae happened quite likely at the end of Miocene, during the salinity crisis of Mediterranean, when they pushed forwards, both westwards, along the northern and southern Mediterranean coasts, and eastwards to Mongolia and China.

01-030

THE RANGE OF EGG CAPSULE MORPHOLOGY WITHIN THE PHASMATODEA AND ITS RELEVANCE TO THE TAXONOMY OF THE ORDER

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Within the order Phasmatodea there is a basic egg capsule morphology which defines the order. This shows extensive variation in detailed form.

Some of this is aberration, which can be recognised and excluded from taxonomic considerations. Much is adaptation to egg-laying techniques and egg survival. Nevertheless the range of the egg capsule form both externally and internally is a valuable aid to the classification at sub-ordinal level and below of this group of insects.

In particular this confirms the Timematodea as members of the order and shows some subfamilies such as Necrosiinae to be taxonomically very diverse.

01-029

KARYOTYPICAL PROPERTIES OF THE SHORT-HORN ORTHOPTERANS INSECTS (ORTHOPTERA, CAELIFERA) IN RUSSIA, KAZAKHSTAN, THE CAUCASUS, AND MIDDLE ASIA FAUNA

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Comparative karyological analysis showed that taxonomic diversity of short-horn orthopterans of the former USSR fauna combines with karyotypical conservatism on the level of large superspecies taxa: Eumastacidae (Gomphomastacinae: $2n=19, 21$; $NF=21-42$), Tetrigidae ($2n=13$; $NF=13$), Arididae ($2n=16, 17, 19, 21, 22, 23$; $NF=21, 23-25, 28$), Pyrgomorphidae ($2n=19$; $NF=19$), and Pamphagidae ($2n=18, 19$; $NF=19$).

On the basis of karyotypes features and patterns of chromosome evolution the scheme of phylogenetic relations of researched groups were made.

01-031

THE ROLE OF VITELLOPHAGES DURING YOLK UTILIZATION IN STICK INSECT EMBRYOS.

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Yolk utilization entails proteolytic degradation of vitellin polypeptides into progressively smaller products. The role played by vitellophages in this process has been investigated by ultrastructural analysis of yolk degradation in the stick insect *Carausius morosus*.

During the first stages of embryogenesis vitellophages invade the yolk fluid mass from the eggs periphery using slender cytoplasmic filopodia. This causes the yolk mass to be gradually engulfed inside the vitellophages and to be partitioned into large intracellular granules. In *C. morosus* the vitellophages originate mainly from a thin layer of stem cells, the so-called yolk cell membrane, interposed between the germ band and the yolk mass. Throughout development, a thin basal lamina separates the yolk cell membrane from the overlying embryo. Later in development, endodermal cells migrate lengthwise along the embryo's axis, gradually replacing the yolk cell membrane.

After yolk sac closure, the intestinal cells start to differentiate. At the posterior end of yolk sac, endodermal cells migrate in clusters spaced out by some intervening extracellular channels. Through these channels the yolk sac lumen becomes continuous with the hemocoel across the basal lamina. As respect to other regions of yolk sac, the vitellophages occupying the posterior end are the first to undergo degradation.

The role of cell-to-yolk and cell-to-cell interactions in the yolk sac are discussed in relation to the process of yolk utilization.

01-032

GENOMIC DNA ANALYSIS OF PARENTAL AND HYBRID *BACILLUS* STICK-INSECTS (PHASMATODEA).

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Genomic DNAs of the bisexual *B. rossius* and *B. grandii* and of their diploid hybrids *B. rossius-g. grandii*, *B. rossius-g. benazzii* and *B. whitei* (= *B. rossius/grandii grandii*) have been tested for the presence of the highly repetitive DNA family *Bab300* (Mantovani et al., Ins. Mol. Biol. 2:141-147, 1993). Multimeric ladders of the monomer were obtained with *AluI*, *TaqI*, *BglII*, *Clal*, *DraI*, *EcoRI*, *NsiI* and *NdeI* restriction enzymes from the genomic DNAs of *B. grandii* subspecies (*B. g. grandii*, *B. g. benazzii*, *B. g. maretimi*) and of the hybrids, whereas they were not observed in genomic digests of *B. rossius*. Southern blots tested with *B. g. grandii* DIG-probes revealed cross-hybridization with digests of the other *B. grandii* subspecies and of hybrid populations. No signal was found in *B. rossius* digests. *In situ* hybridization showed a pericentromeric localization of the *Bab300* family on all chromosomes of *B. grandii* and on the *grandii* haploset embodied in its hybrids (Tinti and Scali, this Congress). Sequence data support the existence of a unique satDNA family in *B. grandii* subspecies and in their hybrids with *B. rossius*. The sequence homology percentages ranged from 80 to 97%, including *B. atticus* sequences. We therefore propose to re-name this satDNA *Bag320*. Dendrograms obtained with different tree-building methods constantly showed a subspecies clustering pattern for *B. grandii* populations; sequences from hybrid strains clustered with the corresponding parental *B. grandii* subspecies. These data support the existence of two well differentiated species-groups (i.e. *B. atticus-grandii* and *B. rossius*) and reinforce the ascertained phyletic relationships between hybrids and ancestral species; moreover, sequence analyses seem to suggest a different evolutionary rate of satDNA in bisexual and parthenogenetic taxa.

01-034

EGG MATURATION AND DEVELOPMENT IN HEMICLONAL AND CLONAL HYBRIDS OF *BACILLUS* STICK INSECTS

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Interspecific crosses between *B. rossius* and *B. grandii* produced two hybridogens (*B. rossius-grandii benazzii*, *B. rossius-grandii grandii*), a diploid parthenogen (*B. whitei*) and, by hybridization to a third species (*B. atticus*), a trihybrid triploid parthenogen (*B. lynceorum*). During hybridogenetic oogenesis unassorted parental genomes are segregated and the paternal *grandii* haploset is eliminated, whereas the *rossius* hemiclone is passed to the offspring. Their hybrid structure is renewed each generation through real fertilization of the hemiclonal eggs by host *grandii* males. The oogenetic mechanism of the corresponding clonal hybrid *B. whitei* is similar, only differing in the automictic re-use of the segregated, not degenerating *grandii* haploset; therefore *B. whitei* offspring maintain the maternal hybrid structure. The triploid *B. lynceorum* produces clonal descendants through an apomictic mechanism undergoing two mitotic divisions. Each *Bacillus* hybrid thus realizes a different egg maturation mechanism, but all of them share an intrameiotic DNA extradoubling leading to fourstranded chromosomes which divide mitotically once (in hybridogens and in *B. whitei*) or twice (in *B. lynceorum*). Finally, fertilized eggs of *B. whitei* are able of incorporating an additional *B. grandii* or *B. rossius* genome, with the production of fertile "synthetic" triploids, embodying two *grandii* and one *rossius* genomes or vice versa. Cytological findings provide evidence that in *Bacillus* allodiploids whole genome segregation, elimination or addition occur. Furthermore they suggest that parthenogenesis may have evolved from hybridogenesis and that unisexuals can keep interacting with related bisexual taxa.

01-033

VITELLIN PROCESSING IN OVARIAN FOLLICLES AND IN EMBRYOS OF *CARAUSIUS MOROSUS* (BR.) (PHASMATODEA: LONCHODINAE)

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Vitellogenins (Vg) are high molecular weight glycoproteins that are transferred from the maternal hemolymph to the oocyte, as vitellins (Vt), during oogenesis. With the onset of embryonic development, Vt are gradually degraded by limited proteolysis to provide raw material for the growing embryo. Vg are synthesized by the fat body, secreted into the maternal hemolymph and specifically endocytosed by the oocyte. In the stick insect *Carausius morosus* transfer through these compartments entails an extensive processing of some Vg polypeptides. Vg processing proceeds *pari passu* with fusion of the yolk granules in the oocyte such that, by the end of vitellogenesis, mature ovarian follicles comprise a unique yolk fluid mass. In this study we have documented the nature and the time course of this vitellogenic protein processing, using specific monoclonal antibodies against Vt polypeptides. Following egg laying, embryos start to develop parthenogenetically. Vitellogenes have already been shown to take part in this process by partitioning the unique yolk fluid mass into a collection of morphologically different yolk granules. Vt are once again processed proteolytically yielding lower molecular weight polypeptides with a stage and time specific appearance. In this study we have documented the pattern of Vt proteolytic degradation and have also identified the peptide fragments that are generated by each Vt polypeptide during embryonic development. In a parallel series of experiments, methacrylate sections of stick insect embryos belonging to different developmental stages were tested by immunostaining using different monoclonal antibodies. The evidence obtained through this procedure shows that yolk granules are differentially stained in relation to the stage of development attained and to the antibody used. The observations carried out in this study suggest a model whereby proteolytic processing of Vg and Vt in stick insects can be functionally correlated with the morphological changes that characterize the yolk granules during vitellogenesis and embryogenesis.

01-035

THE GENUS *SIPYLOIDEA* BRUNNER IN AUSTRALIA (PHASMATODEA: PHYLLIIDAE; NECROSCIINAE)
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The stick insect genus *Sipyloidea* Brunner von Wattenwyl is represented in Australia by at least 12 taxa most of which are undescribed. Elsewhere the genus is represented in south-east Asia and the Pacific with the type species, *S. sipylus* Westwood, having come from Assam. The species are very similar in overall appearance. Males are winged and capable of flight; females are micropterous and bear small, lanceolate wings that are incapable of flight. The body colour is typically light to dark green; rarely the top of the head and the base of the legs may have an orange tinge. In both sexes the tegmen is a small round pad with a dark brown or black patch dorsally, with or without green spots. The male wing has a light grey-green border with the remainder of the wing pink to purple; the minute female wing is grey or greenish grey.

Australian *Sipyloidea* species are difficult to distinguish morphologically. The genitalia provide the best anatomical characters with the shape of the cercus, vomer and tenth tergite being distinctive among males. Females are almost indistinguishable and with sympatry and parthenogenesis common in the genus, museum specimens of the female sex cannot be determined with certainty.

01-036

IN SITU HYBRIDIZATION OF HIGHLY REPETITIVE DNA IN *BACILLUS* STICK INSECTS: CYTOTAXONOMY AND GENOME EVOLUTION

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Systematic investigations on *Bacillus* stick insects allow the recognition of three species groupings within the genus: a) the *grandii-atticus* group, including the bisexual *B. grandii* and the parthenogenetic *B. atticus*, which share most morphological, chromosomal and allozymic features; b) the monospecific *B. rossius* group, showing a great differentiation from the former; c) the 'hybrid' group, constituted by three different *rossius/grandii* diploid hybrids and by one *atticus/rossius/grandii* allotriploid taxon (Scali et al. 1995, Boll. Zool. 65:59).

Molecular investigations on satellite DNA have been recently carried out on all species of the genus using recombinant DNA techniques, sequencing and fluorescent *in situ* hybridization (FISH). Main achievements are: i) the occurrence of the *Bag320* satDNA family in the genome of *B. atticus*, *B. grandii* and their hybrids and its absence from *B. rossius*. DNA sequences from all investigated taxa were always highly homogeneous, but a clearcut cluster differentiated *B. atticus* from *B. grandii* even using different phylogenetic tree-building methods (Mantovani & Tinti, this congress); ii) the *Bag320* satDNA was always localized in the pericentromeric region of the chromosomes; iii) while in the *B. atticus* haplotype (n=17) only a chromosome subset (13-15) showed the hybridization sites, in *B. grandii* all chromosomes (n=17) had the signal; iv) diploid *rossius/grandii* hybrids (2n=35) constantly showed 17 fluorescent spots, corresponding to the *grandii* haploset; v) in the allotriploid *atticus/rossius/grandii* trihybrid (3n=52), 32-34 chromosomes hybridized, corresponding to the *grandii* and the *atticus* haplosets.

Main achievements of these investigations are the cytomolecular characterization of the karyotypes of all *Bacillus* taxa and the recognition that unequal cross-over mechanisms had to work to quantitatively differentiate the *Bag320* family within *B. atticus*.

01-038

SYSTEMATICS, PHYLOGENY, AND A NATIONAL COLLECTION, II: UTILIZING A NATIONAL COLLECTION FOR SYSTEMATICS RESEARCH, THE LEPIDOPTERISTS OF THE UNITED STATES NATIONAL MUSEUM, 1890-1940

M. Epstein

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The National Entomological Collection served as the basis for a strong research tradition in Lepidoptera in the U.S. Lepidopterists such as H.G. Dyar, A. Busck, C. Heinrich, W. Schaus, and J.F.G. Clarke both utilized and continued to develop the national collection of Lepidoptera for research in insect systematics and evolution and economic entomology. The close working relationship between entomologists at the United States Department of Agriculture and the United States National Museum allowed the national collection to grow during a period when the museum provided minimal or no staffing. This era was characterized by colorful personalities who engaged in legendary debates with their colleagues within and outside the museum. Issues such as the development of the International Code of Zoological Nomenclature, the admissibility of Huebner's *Tentamen*, and the proposed sale of the Barnes collection united previous adversaries. At the end of this era, Clarke began his career with the USDA. He was later to transfer to the Smithsonian and, through his leadership, found a separate Department of Entomology, increasing the staffing of entomologists at the museum to far beyond previous levels.

01-037

SYSTEMATICS, PHYLOGENY, AND A NATIONAL COLLECTION, I: THE ROLE OF C.V. RILEY, J.H. COMSTOCK, AND L.O. HOWARD IN CREATING A NATIONAL ENTOMOLOGICAL COLLECTION IN THE UNITED STATES

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The National Entomological Collection at the National Museum of Natural History, Smithsonian Institution, (formerly the United States National Museum) is the result of the vision of three seminal figures in the history of entomology in the United States: Charles Valentine Riley, John Henry Comstock, and Leland Ossian Howard. Riley established a national entomological research and collecting program at the United States Department of Agriculture. That collection became the core of the National Museum collection. Comstock advocated a national collection for basic systematic and evolutionary research, as well as economic entomology. His student, Howard, synthesized the approaches of Riley and Comstock, as he served simultaneously as Chief of the USDA Bureau of Entomology and Honorary Curator of the Division of Insects at the National Museum. As the Smithsonian celebrates its 150th anniversary in 1996, this session will trace the history of its role in the development of American entomology.

01-039

THE COLLECTION OF TYPHLOCYBINAЕ BY CARLO VIDANO (RHYNCHOTA AUCHENORRHYNCHA)

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Insect collections have an important role in systematics because they offer a precious material of comparison for the classification of the entomofauna. Collections, even of small entity, but of a high specialization, have a special significance when they are made by entomologists who, besides the locality, consider environment, biotope, host plant, niche, localization on the plant, trophic behaviour, and so on.

In this frame, the collection of European Cicadellidae Typhlocybinae by Carlo Vidano has a noteworthy interest for the specialists of these insects. It comprises about 16,000 specimens of 160 species collected systematically in all Italian regions and desultorily in many European countries, including the types of the 12 species described by Vidano, and is arranged in 55 entomological boxes.

The specimens were collected rarely by means of an entomological net, more frequently with a glass tube on well known herbaceous plants or trees; several of them emerged from nymphs reared in captivity on the host plants. The most part is preserved dry on pointed cards or stored in small plastic tubes. Since the classification of these species is made by means of the analysis of male genitalia, several males of each species are dissected and their anal block and aedeagus are prepared and glued on the same card. The number of the collecting protocol together with locality, altitude, and host plant are reported on cards underneath.

The collection is completed and made precious by the collecting protocols, which are an integrant part of it and are gathered in 5 note books. 974 protocols are written in these books in a chronological order, and concern the captures carried out from 1955 to 1989. For each locality they provide altitude, exposition, date, hour, biotope, vegetal biocenosis, reproduction site, natural enemies, meteorological conditions, insolation, shading, and any element useful to the knowledge of ecological and ethological aspects of the different species.

01-040

NOTES ON THE CONTEMPORARY CLASSIFICATION OF DERMAPTERA

S.SAKAI, Institute of Life Sciences, Daito Bunka University, No.2-26-12, Sendagi, Bunkyo, Tokyo, Japan:113.

The author presents a new revisional classification of Dermaptera and all known species and Fossil species of Dermaptera are listed up until July, 1995. There are 4 suborders, 4 superfamilies, 11 families, 54 subfamilies, 11 groups, 3 tribes, 219 genera, 40 subgenera, 1,951 species, 37 subspecies, 34 varieties and 13 forma of Dermaptera of the world including 55 fossil Dermaptera. Arixeniina: 2 genera and 5 species; Hemimerina: 3 groups, 9 species and 1 variety; Pygidicranoidea: 2 families, 12 subfamilies, 8 groups, 30 genera, 11 subgenera, 361 species, 3 subspecies, 2 varieties and 2 forma; Anisolabidoidea: 2 families, 11 subfamilies, 45 genera, 16 subgenera, 418 species, 10 subspecies, 2 forma and 7 varieties; Apachyoidea: 1 family, 1 subfamily, 2 genera and 15 species; Spongiphoridae: 13 subfamilies, 3 tribes, 42 genera, 504 species, 12 subspecies, 6 varieties, and 4 forma; Chelisochidae: 2 subfamilies, 15 genera, 95 species, 1 subspecies and 1 variety; Forficulidae: 10 subfamilies, 65 genera, 13 subgenera, 486 species, 11 subspecies, 17 varieties and 5 forma; Fossil Dermaptera (excluding Protelytroptera): 7 families, 28 genera and 55 species. In Pygidicranidae, Acrania and Picrania are valid genera. Cranopygia is separated into 3 groups: Cumingi, Marmoricrura and Valida groups. Prolabiscinae is valid subfamily. The genera in Diplatyinae: Haplodiplatys, Paradiplatys, Circodiplatys, Nannopygia, Diplatys; Gonolabina transferred to Esphalmininae. In Forficulinae; Chelidura is separated into 6 subgenera: Chelidura, Borelliola, Burriola, Maraniola, Mesochelidura and Chelidurella.

01-042

DERMAPTERAN CENTRES OF EVOLUTION.

Edward J. Popham, 26 Branch Rd. Mellor, Blackburn, England.

and Seiroku Sakai, Institute of Life Sciences, Daito Bunka University, Tokyo, Japan, 113. The analytical techniques of Newton & Platnick (1981) have been applied to Sakai's phylogenetic trees to deduce the centres of evolution of the main Dermapteran taxa with special reference to their geographical distribution on the land masses before the opening of the Pacific Ocean in the Triassic. The Pygidicranidae, Anisolabidae, Arixeniidae and Labiidae originated in the same equatorial region of the eastern Pacific, whereas the Labiduridae, Apachyidae, Chelisochidae and Forficulidae have common Afro-Indian centres of evolution. The Hemimerina are of African origin. Sakai's classification suggests that his two Infraorders each have the same two widely separated centres of evolution: an anomaly which Popham's (1965, 1983 and 1990) classification of the Order avoids by placing the Anisolabidae, Labiidae and Arixeniidae in one taxon and the Labiduridae, Chelisochidae, Apachyidae and Forficulidae in another. The African Hemimerina were placed in a separate but allied order.

01-041

THE PHYLOGENY OF THE FORFICULINA (DERMAPTERA)

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The phylogeny of the Forficulina (Dermaptera) has been reassessed. Forty-eight species and thirty characters were examined, of which thirteen characters of thorax and wings are described or used for phylogenetic purposes for the first time. The remaining seventeen characters have been extracted from literature. Examination of the thirty characters demonstrates that only twenty-three characters are useful for phylogenetic reconstruction. The characters have been analysed with PAUP 3.1 yielding two equally parsimonious trees. The results suggest an exclusion of the 'Diplatyidae' (themselves paraphyletic) and the Karschiellidae from the Pygidicranidae and support the separation of the Apachyidae from the Labiduridae. A sister-group relationship of the Anisolabidae and Spongiphoridae is not supported. The monophyly of the Eudermaptera is confirmed.

The relationship is:

(Karschiellidae ('Diplatyidae' (Pygidicranidae (Apachyidae (Labiduridae (Anisolabidae (Spongiphoridae (Chelisochidae, Forficulidae)))))))).

The results demonstrate that a re-interpretation of the evolution of the male genitalia is necessary. The last common ancestor of the extant Forficulina had genitalia with two lobes pointing in one direction, with two virgae each. This type of genitalia is found in the Diplatyidae. So, it represents a plesiomorphy and not an autapomorphy as assumed before. As the next stage, one virga in each lobe was reduced, resulting in a situation which is found in the Pygidicranidae. However this is not an autapomorphy for this family. After that, one lobe changed direction, pointing cranially now. It is not a synapomorphic feature of the Apachyidae, Labiduridae and Anisolabidae, as shown by the branching pattern; it is a „stage“ in the evolution. Finally, the number of lobes was reduced to one. This situation is found in the Spongiphoridae, Chelisochidae and Forficulidae and is considered as a synapomorphy for these groups.

01-043

DERMAPTERA AND PROTELYTROPTERA - ONE ORDER?

A.V. GOROCHOV (Zoological Institute, Russian Academy of Sciences, S. Petersburg, 199034)

L.N. ANISYUTKIN (S. Petersburg University, Russia)

S.SAKAI, Institute of Life Sciences, Daito Bunka University, No.2-26-12, Sendagi, Bunkyo, Tokyo, Japan:113.

The orthopteroid orders Orthoptera, Dictyoptera (Blattodea+Mantodea), Plecoptera, Grylloblattida, Phasmoptera have very long and rich history (from Palaeozoic). Sometimes these orders are presented by 2 or more recent stocks diverging from Palaeozoic or Triassic (for example Orthoptera). These stocks we usually consider as suborders or infraorders. Sometimes the orders are presented only by 1 recent stock of the same age (for example Dermaptera). Nevertheless this stock usually also subdivides into suborders and infraorders (inequivalent to the analogous taxa of previous orders as the rate of evolution of different orders of orthopteroids was more or less similar except maybe Isoptera).

Dermaptera sensu stricto (without Archidermaptera and enigmatic Hemimerina) appear from late Jurassic. Their morphological diversity is almost equal to that of the superfamily Grylloidea (from late Triassic or early Jurassic). On the other hand the Permian Protelytroptera is undoubtedly ancestral group for recent earwigs (all peculiarities of skeleton and wing venation confirm this origin). Therefore we propose to consider the Protelytroptera as a suborder of Dermaptera (sensu lato) and to consider the Dermaptera sensu stricto (+Archidermaptera) as other suborder. This conception allows to lower the ranks of other higher taxa of the order. In that case order Dermaptera becomes comparable with other large orders of orthopteroids by age and level of differentiation.

01-044

MIT.DNA, BIOSYSTEMATICS AND EVOLUTIONARY BIOLOGY OF EARWIGS.

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URA-CNRS 1853, Université de
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First attempts of between families comparison of earwig mit. DNA, revealed particular base composition in the order. This new parameter, as well as the genetic distances between different families (Forficulidae, Labiduridae, Carcinophoridae) evolves consistently with the phylogenetical reconstructions established on morphological characters (Popham, 1965; Sakai, 1986).

These phylogenetical relationships also agree with the tropical origin of the order associated with iteroparity and the « apomorphic » status of adaptations to colder climates, leading to semelparity repeatedly observed in alpine and continental conditions.

01-046

BIOCHEMICAL ANALYSIS IN GEOGRAPHICAL VARIATION OF *ANECHURA HARMANDI* (FORFICULIDAE, DERMAPTERA)

TERARA, Kohji and SAKAI, Seiroku (Institute of Life Sciences, Daito Bunka University, Higashimatsuyama, Saitama, Japan 355)

Anechura (Odontopsalis) harmandi (Burr, 1904) had three morphological forms, that is, forma *harmandi*, *lewisi* and *soedaensis*. In Honshu or the Main Island of Japan, there are two types of population, which are *lewisi*- monomorphic populations and *lewisi* & *harmandi*-polymorphics.

Three monomorphic populations and nine polymorphics were electrophoretically compared with each other in three enzymes of Mdh(1 locus), 6Pgdh(1 locus) and G6pdh(2 loci). Nei's distances between populations were summarized by UPGMA. As a result, the monomorphic and polymorphic populations were not distinguished and the difference between them was small except for two populations of monomorphic and polymorphic ones. The two were over subspecies- level distant from the others.

Furthermore, the monomorphic and polymorphic populations were compared with each other by means of random amplified polymorphic DNA-polymerase chain reaction.

01-045

ONE NAME BUT TWO SPECIES: MOLECULAR PHYLOGENY AND BIOGEOGRAPHY OF THE SO CALLED "EUROPEAN EARWIG", *FORFICULA AURICULARIA*.

T. Wirth¹, R. Le Guellec²,
M. Vancassel¹, M. Veuille³.

1, URA-CNRS 1853 and 2, URA-
CNRS 256, Université de Rennes 1, France;
3, Institut d'Ecologie,
Université ParisVI, France.

The discovery of a very distinct reproductive isolation between two populations of *F. auricularia* led to investigation of possible genetic divergence. A fraction of mit. DNA was analysed for fifteen European and North American populations. Results of between populations crosses were confirmed by measurements of genetic distances which indicated the existence of two distinct lines. The *F. auricularia* taxon in fact includes two twin species. Their contemporary distribution reflects their long standing presence in Europe and multiple recent introductions to North America.

01-047

KARYOTYPE ANALYSIS OF A FORFICULID EARWIG, *ANECHURA HARMANDI* (FORFICULIDAE, DERMAPTERA)

HOSHIBA, Hidehiro and SAKAI, Seiroku (Daito Bunka University, Dai-ichi High School, Takashimadaira, Itabashi, Tokyo, Japan 175 and Institute of Life Sciences, Daito Bunka University, Iwadono, Higashi-Matsuyama City, Saitama, Japan 355)

The chromosomes of a Forficulid earwig, *Anechura harmandi* (Burr, 1904) were observed in the male germ cells.

Materials were collected at Okuchichibu, Saitama, Japan on March, 1985. Their testes were dissected out in the 1% sodium citrate solution containing 0.001% colchichin and kept in this solution for 30 min. Preparations were made by lactic acid dissociation, drying technique improved by Hoshiba et al.(1993). Twenty nine cells from five adult males were analyzed. Their chromosome number was $2n=25$.

Although 487 Forficulid species have been recorded, only 11 species of their chromosomes have been studied. Their number of autosome pairs (N^{ap}) are mostly eleven and they are characterized by sex chromosomes polymorphism such as $N^{ap}=11$ with XY (*Anechura bipunctata*, *Apterygida media*, *F. tomis succuderi*, *F. beelzebub*, *F. schlagintweitii*, *F. mikado* and *Forficula auricularia* has XY₁, XY₂), X₁X₂Y (*Pseudochelidura sinuata*) and XY₁Y₂ (*F. auricularia*). The others are the species with $N^{ap}=9$ (XY, *Elaunon bipartitus* and X₁X₂Y, *F. smyrnensis*) and $N^{ap}=7$ (XO, *Allodahlia macropyga*).

The chromosomes of *Anechura harmandi* probably consisted of 22 autosomes and X₁X₂Y. Further chromosome observations on spermatogenesis and female oogonial cells are required to determine the sex chromosomes.

01-048

KARYOTYPE EVOLUTION IN DERMAPTERA

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and

S. SAKAI, Institute of Life Sciences, Daito Bunka University, No.2-26-12, Sendagi, Bunkyo, Tokyo, Japan : 113

Survey of the cytologically known dermapterans reveals that their chromosome numbers vary from $2n=7$ in *Hemimerus bouvieri* to $2n=42$ in an unidentified species of *Anisolabis*, with gaps at certain numbers. In species with $2n=10-20$ two categories of chromosomes exist viz. short rod- and large rod-like. In another group of these insects with $2n=20-30$, moderate-sized chromosomes are met with. However, in forms with $2n=32$ or more, the chromosomes are small rod-like. The co-relation between the numerical decrease of chromosomes and relative increase in their length or vice-versa strongly supports that the karyotype evolution in Dermaptera has taken place either by fusion of the smaller chromosomes or by fission of larger ones, resulting in numerical decrease with increased length or numerical increase with decreased length of chromosomes, respectively. It seems, this type of evolution has been facilitated by the presence of diffused or at least non-localised nature of the kinetochore in these insects, since it renders the chromosome fragment or fused chromosome easy to function as a autonomous whole, as is true of other primitive insects like aphids and bugs etc.

01-050

A PREVIEW OF THE CATALOGUE OF THE DERMAPTERA OF SPAIN

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The latest studies made on the Fauna of Dermaptera in peninsular Spain and the Balearic Islands comprise 22 species including such endemic as *Eulithinus analis* (Rambur), *Mesochelidura bolivari* (Dubrony), *Pseudochelidura sinuata* (La Fresnaye) and *Chelidura pyrenaica* (Gené).

Canary Island Fauna include the following endemic species: *Anataelia canariensis* Bolívar, *Anataelia lavicola* Martín and Oromí, *Anataelia troglobia* Martín and Oromí, *Anisolabis maxima* (Brullé), *Perirhytus endetelus* (Woll.) and the ten species of the genus *Guanchia* (*G. bandamaensis* Morales, *G. cabreræ* (Bolívar) *G. canariensis* (Burr), *G. fernandesi* Morales, *G. gomerensis* Morales, *G. guanchia* (Heller), *G. storai* (Chopard), *G. tenerifensis* Morales, *G. transversa* Brindle and *G. uxoris* Heller. The Catalogue of the Dermaptera of Spain comprises 37 species (3 Pygidicranidae, 7 Carcinophoridae, 2 Labiduridae, 1 Labiidae and 24 Forficulidae), 2 varieties and 2 forms.

01-049

Notes on four new species of Dermaptera from China.

Zhou, Wen-bao (Zhejiang Museum of Natural History, Hangzhou, 310012) and Sakai, Seiroku (Institute of Life Sciences, Daito Bunka University, No.2-26-12, Sendagi, Bunkyo, Tokyo, Japan 113)

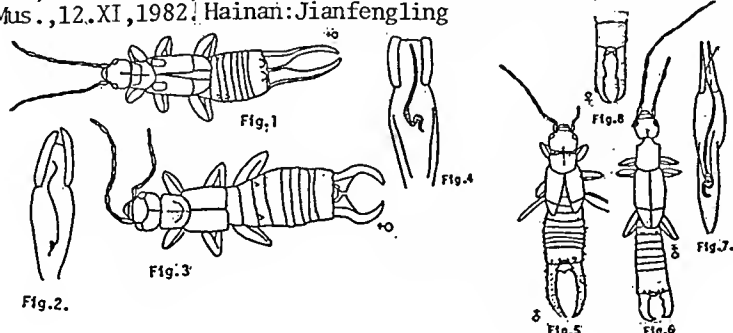
4 new species: *Forficula bimaculata* Zhou et Sakai, Figs.1-2; *Forficula ruiliensis* Zhou et Sakai, Figs.3-4; *Anechura sakaii* Zhou, Fig.5; *Auchenomus huai* Zhou et Sakai, Figs.6-8.

Forficula bimaculata Zhou et Sakai: Male, Length of body with forceps: 16 mm. Tegmina brownish red with a yellow oval spot at the shoulder, Genitalia: virga forming an angle at the junction with the basal vesicle. Holotype & Paratype male: Zhejiang Mus., 20.VI.1987, Fengangshan, Zhejiang.

Forficula ruiliensis Zhou et Sakai: Body length: Male: 8.5-10.5, Female: 11.0 mm; Forceps: Male: 2.8-3.0, Female: 2.9 mm. Zhejiang Mus., 5.VI.1983, Yunnan: Ruili

Anechura sakaii Zhou: Body length: Male: 8.5 mm; Forceps: 4.0 mm. Although Sakai (1995: Dermapt. Cat., 26: 5951, 27: 6946, 7051) synonymized with *Anechura pilosa* Ma et Chen (1992), Now this species is valid species. Male: Holotype: Zhejiang Mus., 10.VII.1986. Zhejiang: Longquan, Fengyangshan.

Auchenomus huai Zhou et Sakai: Body length: Male 10.0, Forceps 2.0; Female: 12.0, Forceps: 3.8 mm. Holotype, Allotype: Zhejiang Mus., 12.XI.1982, Hainan: Jianfengling



01-051

RESEARCHES ON PHILIPPINE DERMAPTERA

S. SAKAI, P. A. JAVIER and C. D. ABELLON; Institute of Life Sciences, Daito Bunka University, No. 2-26-12 Sendagi, Bunkyo, Tokyo, Japan: 113; National Crop Protection Center, WPLB, College, Laguna; RDC-JKL, #12 General Macundo St., Baguio City, Philippines, respectively.

The present record of Philippine Dermaptera consists of 145 species belonging to two suborders, 18 subfamilies and 46 genera.

Some of the dominant species of earwigs that play a major role in the biological control of the Asian corn borer, *Ostrinia furnacalis* were *Euborellia annulata*, *E. philippinensis*, *Proreus simulans*, *Nala lividipes* and *Labiduria riparia* in decreasing order. The life cycle of these five earwig predators were discussed. The predators were reared in the laboratory using larvae and pupae of red flour beetle, *Tribolium castaneum*.

The dispersal of adult *E. annulata* was studied by release and recapture of marked individuals. The percentage recovery was higher during the initial sampling and then decreased in the succeeding samplings. The large percentage recovery was observed at a distance of three to six m from the release point. As the distance traveled by the earwig from the release point increased, the recovery decreased. The initial maximum distance traveled by the earwig after release was six m and then increased to 9.5 m after 14 days. Based on simple linear regression analysis, the dispersal of earwig consistently increased with time after release. The dispersal pattern was found to be clumped.

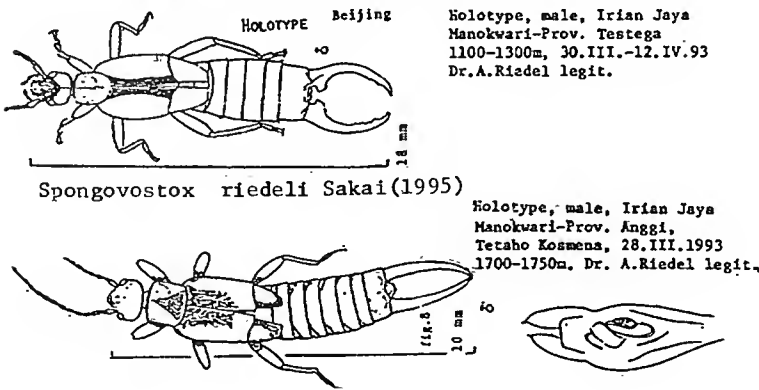
The selectivity to the earwig of eight insecticides (recommended against corn borer) of different chemical structures were evaluated. The adverse effects of the insecticides to the earwig decreased as follows: monocrotophos > deltamethrin > cypermethrin > chlorpyrifos ethyl + BPNC > carbofuran > methomyl = carbaryl > endosulfan. Based on the selectivity ratio, carbofuran and cypermethrin are the most selective insecticides to the earwig.

01-052

NEW Records of New Guinean Dermaptera,

SAKAI,Seiroku,Institute of Life Sciences,Daito Bunka University,No.2-26-12,Sendagi,Bunkyo,Tokyo,Japan,113. and Zhang,Xiao-chun',Institute of Zoology,A'camia Chinica, Beijing,19 Zhongguancun Lu,Haidian,Beijing 100080,China.
22 species of Dermaptera from New Guinea as the result of the expedition of New Guinea by A.Riedel,1990-1993.
Adiathetus papuaensis Sakai(1995:Dermapt.Cat.30:9303)
Chelisochinae,Chelisochidae,Irian Jaya 1100-1300 m and *Spongovostox riedeli* Sakai(1995;Dermapt.Cat.30:9306)
Spongiphorinae,Spongiphoridae,Irian Jaya,1700-1750 m are described.

Systematic records of New Guinean Dermaptera:
Anisolabididae:*Euborellia plebeja*,*Gonolabis javana*,Apachy-
dae:*Apachyus becaarii*,Spongiphoridae:*Nesogaster aculeatus*,
Auchenomus blumi,*Auchenomus setulosus*,*Chaetospania fuscata*
fuscata,*Paralabella fruhstorferi*,*Paraspania brunneri*,*Irdex*
ernstmayri,*Spongovostox riedeli*,Chelisochidae:*Chelisoches*
ater,*Chelisoches morio*,*Adiathetus papuaensis*,Forficulidae:
Acanthocordax papunus,*Acanthocordax rhachynotus*,*Spinoso-*
cordax excelsior,*Hypurgus biroii*,*Pterygida burgursi*,*Ptery-*
gida poultoni. *Adiathetus papuaensis* Sakai(1995)



01-054

A PHYLOGENETIC CLASSIFICATION OF THE APACHYIDAE (DERMAPTERA) AND BIOGEOGRAPHIC ANALYSIS
G.CASSIS

The Apachyidae are defined as a monophyletic family of Dermaptera on the basis of characters of the mouthparts, abdomen and genitalia. The family contains three genera and fifteen species. A new genus is erected for the annectant species, *Dendroiketes novaeguineae* Boeseman. A new species of *Apachyus* Audinet-Serville is described from the Northern Territory of Australia and the peculiar morphology of *Apachyus peterseni* Borelli is described in detail. A cladistic analysis of the species will be presented with emphasis on the biogeography of this palaetropical group. The area relationships will be discussed in relation to current models of Indo-Pacific biogeography (Schuh and Stonedahl, 1986). The systematic position of the Apachyidae will be discussed in view of the current polemics of their relationships (Popham, 1965; Steinmann, 1975; Haas, 1996).

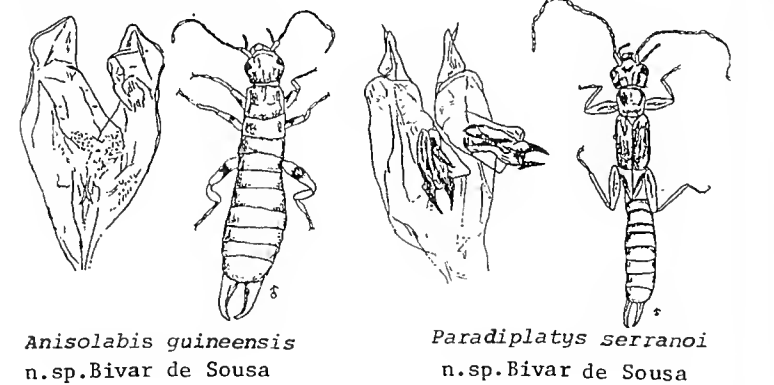
01-053

CONTRIBUTION TO THE STUDY OF EARWIG(INSECTA,DERMAPTERA) FROM GUINÉ-BISSAU.
A.Bivar de Sousa,Sociedade Portuguesa de Entomologia, Praceta Fernando Pessoa,3-9°Esq.Carnaxide,P-2795 Linda a Velha Portigal and Seiroku Sakai,Daito Bunka University, No.2-26-12,Sendagi,Bunkyo,Tokyo,Japan 113.

The Guine-Bissau(Formerly Portuguese Guiné)Dermaptera were firstly studied by Borelli(1907)based on Leonardo Fea collected in this territory between 1898 and 1900.In that work a total of 14 species were recorded. Some up-dates concerning taxonomic nomenclature or synonymies and based always in Borelli(op.cit.)work were published by Rehn(1924], Hincks(1951,1955) and Brindle(1973,1978).

This paper gives faunistic and ecological informations about the Dermaptera fauna of Guiné-Bissau,based on field research during the following periods:16,November-7,December 1983,14,June-5 July,1992 and 25 June-17 July,1995.

From this field research 17 species were identified and among them 10 are recorded for the first time for Guiné-Bissau being 2 of them new species (*Anisolabis guineensis* n.sp.Bivar de Sousa and *Paradiplatys serranoi* n.sp.Bivar de Sousa). With this study a total of 24 species are reported for this country.



01-055

ON THE PHYLOGENY OF ADEPHAGA (COLEOPTERA), WITH SPECIAL EMPHASIS ON TRACHYPACHIDAE

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Edges of rivers are a likely habitat of the stem-species of Adephaga. The ability to survive in an environment which is flooded periodically was an important requirement for early representatives of the suborder. Aquatic habits were acquired three times independently in the evolutionary history of Adephaga. This hypothesis is based upon the phylogenetic interrelationships of the families and the different adaptations of the three aquatic monophyla. Gyrinidae were the first group to enter the aquatic environment. A sistergroup relationship between this taxon and the remaining Adephaga is well supported by larval and adult features. A second and third invasion was accomplished by Haliplidae and Dytiscoidea respectively. Trachypachidae, which are the sistergroup of Dytiscoidea or Carabidae, were widespread in the early Mesozoic. The monophyly of the genera *Trachypachus* and *Systolosoma* is well supported by apomorphic character states of larvae and adults. Trachypachidae were reduced to a small relict group in the Cretaceous. Extroral digestion and correlated structural features are more advanced in carabid larvae than in those of Trachypachidae. The enormous expansion of Carabidae during the late Mesozoic was probably closely connected with the angiosperm revolution. A correlation between characteristic structural transformations of the pro- and mesothorax and the appearance of leaf litter is likely. Similar apomorphic conditions may have evolved several times independently within Carabidae.

01-056

INFERRING PHYLOGENETIC RELATIONSHIPS WITHIN CARABIDAE FROM CHARACTERS OF THE FEMALE REPRODUCTIVE TRACT

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The female reproductive tract of Carabidae--spermatheca and associated glands, bursa copulatrix, and ovipositor--offer a rich array of characters that are stable or associable via transformation series within lower-level lineages, yet strikingly divergent at the tribal to subfamilial ranks. In order to illustrate the utility of this character system for phylogenetic inference, representatives of 69 of the 75 tribes of Carabidae *sensu lato*, recognized by Erwin's classification, were examined and used as exemplars in a parsimony based cladistic analysis. Characters of abdominal configuration, delineated by Deuve, were also included in the analysis. Where possible, a variety of cladistically basal and derived taxa were examined within each tribe. This approach, by its nature, may result in hypotheses that render tribal taxa para- or polyphyletic, however, as the study's aim is to provide a broad sketch of carabid relationships useful for more intensive studies, such a shortcoming is considered justified. Groups traditionally difficult to place form one focus of the study; Rhysodini, Brachinini, and Pseudomorphini. *Omoglymmius* of Rhysodini exhibits derived states of a vaginal apophysis and subsegmented gonocoxae, suggesting affinities to Metriini+Ozaenini+Paussini. Brachinini possess segmented gonocoxae and a spermatheca with apical reservoir and appendiculate spermathecal gland, consistent with placement in Anisochaeta, though adult characters argue against placement in the Harpalidae of Deuve. Pseudomorphini exhibit a spermatheca with appendiculate gland, triangular laterotergites, and falciform gonocoxae, suggesting placement near Pogonini and Patrobini, consistent with Erwin's placement--based on male aedeagal and larval characters--as a lineage basal to Pterostichini.

01-058

MORPHOFUNCTIONAL AND ECOLOGICAL FEATURES IN CARABID LARVAE: A CONTRIBUTION TO DISTINGUISH BETWEEN AFFINITY AND CONVERGENCE.

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Larval stages of Carabid Beetles show a wider morphological variation than the adults, but the most characters they "offer" are normally used only as character states for reconstructing phylogeny. The authors tried to investigate morphofunctional features of about 60 genera, starting from Sharova's "morpho-ecological types" and including a re-examination of recent literature on head-muscle morphology and preying behaviour. Morphometry of mandibles, shape and size of head capsule and adductory muscles, integumental sclerification and morphology of urogomphi have been correlated to: phytophagous or predatory habits, type of prey (molluscs, earthworms, collembolans, etc.), behaviour, overall relationship to soil features and soil layer, life on vegetation, parasitoid lifestyle.

The most important selection pressures determining morphofunctional adaptation of Carabid larvae seem to be i) soil and ecosystem type, that influence especially body shape and urogomphi; ii) prey selection, that influences mostly the form of the head and of mandibles. A possible evolutionary "pathway" of larval functional morphology is sketched, evidencing some key-selection pressures. The validity of some of the basic morphotypes recognized by Sharova has been confirmed.

Pattern and occurrence of morphofunctional characters in some of the commonly accepted phyletic trees of Carabidae is discussed.

01-057

PHYLOGENETIC INVESTIGATION OF CARABIDAE USING LARVAL CHARACTERS

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Morphological characters of larvae of 44 tribes of Carabidae as well as representatives of Trachypachidae (both genera), Rhysodidae, all families of Hydradephaga, Micromalthidae (Archostemata), and 6 families of Polyphaga are examined. Larval characters suggest the monophyly of Carabidae (excluding Rhysodidae), Trachypachidae and Dytiscoidea. The presence of egg bursters in first instar is a synapomorphy of these taxa. The Carabidae are monophyletic and characterized by 3 possible autapomorphies in larval stage. Larvae of Rhysodidae are extremely derived and lack the autapomorphies of Carabidae.

The position of tiger beetles is not to clarify with larval characters at present. The recent classification of tiger beetles (with two subgroups and 5 tribes) is not confirmed by larval characters. Only a part of Megacephalini are the sister group of the rest of cicindelid taxa. Metriitae and Paussitae form a monophyletic unit, which has separated very early from the rest of Carabidae.

Larval characters support the monophyly of subfamily Harpalinae (*sensu* Erwin) including all "higher" carabids. The larval characters found in Platynini may come close to the ground plan of the Harpalini. A monophyletic unit which comprises all members of the Pterostichini, Morionini, Callistini, Oodini, Licinini, Panagaeini, Peleciini, Harpalini, Cnemacanthini, and Zabrinini is characterized by a significant synapomorphy: a membranous band in the middle of stipes. The monophyly of Truncatipennia (excluding Brachinitae) is not confirmed by larval characters.

01-059

An overview of karyotypic evolution and phylogeny of carabid beetles.

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More than eight hundred species of Caraboidea have been karyotypically studied, mostly from the Holarctic fauna. Data usually refer to basic characters such as chromosome number (diploid or haploid) and type of sex chromosomes. Other characters such as chromosome bands, presence of satellites, or estimation of genome size, have been determined only for a few species. The usefulness of karyotypic data for phylogeny depends also on the karyotypic pattern of each taxa, some of which are rather conservative whereas others show a high dynamism.

The family as a whole shows the highest variation in chromosome number within the Coleoptera, making it difficult to assess the ancestral diploid number. However, it is clear that the suborder Adephaga is characterised by chromosome numbers higher than $2n = 18 + Xyp$ karyotype predominating within the other suborders of Coleoptera. In addition, the typical "parachute" y chromosome of the order is not present within the Adephaga.

A variety of situations is found within between lower taxa, as some show a constancy in karyotypic characters (e.g., genera *Carabus*, *Bembidion*, *Chlaenius*), whereas others show marked differences even between closely related species (e.g., genera *Ceroglossus*, *Nebria*, *Scarites*, *Poecilus*, *Brachinus*).

The main conclusions concerning supraspecific taxa are: 1. Carabini is an homogeneous taxa including both *Carabus* and *Calosoma*, and is related to Ceroglossini; 2. Cicindelids are characterised by multiple sex chromosomes although Megacephalini may have experimented a reversion to simple systems; 3. Scaritini, Clivinini and Dyschiriini are different tribes according to their chromosome number and size; 4. The supertribe Trechitae is characterised by an achiasmatic meiosis, although each tribe shows its own karyotypic formula; 5. Tribes of Pterostichitae share the same karyotypic pattern of high numerical variation around $2n = 37$; the subtribes of Zabrinini show opposite karyotypic patterns; 6. Ditomina is a subtribe supported by high chromosome numbers ($2n = 45-69$) with regard to other subtribes of Harpalini; 7. Within the supertribe Lebiitae a suprageneric taxon joining *Microlestes*, *Lionychus* and *Syntomus* is supported by present data; 8. Brachinina and Aptinina seems to share an ancestral karyotype of $2n = 32$, whereas Pheropsophina is characterised by a different number, $2n = 35-36$; these subtribes shows the same predominant pattern towards low chromosome numbers.

Other phylogenetic inferences concern relationships between related genera or species; some sibling species show marked differences in karyotypic features.

01-060

PHYLOGENY OF CARABID TRIBES AS INFERRED FROM MOLECULAR SEQUENCE DATA (COLEOPTERA)

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The phylogenetic relationships of carabid tribes has been investigated using DNA sequences. This report of work-in-progress focuses on some enigmatic groups within carabids:

- (1) are brachinines related to the ozaenine-paussine complex, or are they members of Harpalinae?
- (2) is *Gehringia* one of the basal stocks of carabids or is it a psydrite?
- (3) to what are *Omophron*, pseudomorphines, and morionines related?

Some questions relating to the composition of Carabidae (including the placement of trachypachids) are also addressed with molecular data.

01-061

KARYOTYPIC EVOLUTION AND MITOCHONDRIAL DNA PHYLOGENY OF THE GROUND BEETLE GENUS *SCARITES* (COLEOPTERA: CARABIDAE) FROM THE IBERIAN PENINSULA

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The chromosomal number, the sex-determining mechanism and the chromosomal localization of ribosomal DNA loci have been determined in the seven Iberian species of the genus *Scarites*. The chromosome number varies between $2n=38+X$ in *S. planus*, and $2n=60+X$, in *S. laevigatus*. Male sex chromosomes are XXY in *S. buparius* and *S. occidentalis*, and XO in the other species. Fluorescence in situ hybridization using a PCR amplified ribosomal probe obtained from *S. occidentalis*, indicates that rDNA clusters have an autosomal position varying from four to six sites in all species except for *S. planus*, in which there are four sites in two autosomal pairs and one site in the single X chromosome. In addition, it has been sequenced a 280 bp fragment of the cytochrome oxidase I gene, and analyzed by maximum parsimony and sequence distance methods. These results suggest the following conclusions: 1. The species of the subgenus *Scallophorites*, *S. occidentalis* and *S. buparius*, are clustered together both by karyotypic and molecular results; 2. The species of the subgenus *Scarites*, *S. eurytus* and *S. hespericus*, are related by karyotypic features, and the mtDNA sequence of *S. eurytus* relates this species to the subgenus *Parallelomorphus*; 3. The two species of the subgenus *Parallelomorphus*, *S. laevigatus* and *S. terricola* are also grouped by both kind of data; 4. The single species of the subgenus *Distichus*, *S. planus*, is the most distantly related to the other Iberian *Scarites* on the basis of the presence of rDNA sites in the X chromosome, and the presence of an A+T rich intergenic spacer of 121 bp between the stop codon of the COI gene and the tRNA^{leu}, which is not present in the other species of the genus. These differences support the proposed ranking of *Distichus* as a separate genus, based on morphological characters.

01-062

THE PHYLOGENETIC RELATIONSHIPS OF CICINDELOIDS: EVIDENCE FROM DNA SEQUENCES

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The Cicindelidae are a group of some 2000 described adephagan species which are generally placed in the vicinity of the Carabidae. Competing hypotheses group the cicindelids either within the Carabidae (near the hiletines, the loricarines, or the carabines) or outside of the Carabidae (as the sister taxon or as a clade separate from the Carabidae retaining many of the ancestral features of early Adephaga). We are using DNA sequences to address the relationships of cicindeloids to each other and to other adephagan groups. Both nuclear and mitochondrial rDNA sequences indicate that cicindelids are very different from other caraboids, and no clear synapomorphies with the major tribes of the Carabidae could be found. The molecular data provided a well supported phylogeny for most of the major groups of cicindelids. Unequivocal evidence against the traditional division of the family into two subfamily (Cicindelinae and Collyrinae) was obtained. Taxa in the tribes Omini and Mantichorini are basal for the group whereas the Collyrinae are embedded within the Megacephalini of previous authors. The genus *Cicindela* (s. l.) is the most derived group of the family. The phylogenetic hypothesis for the Cicindelidae will be discussed with respect to shifts in ecological parameters during the evolution of the lineage.

01-063

WHERE DO THE RHYSODINES FIT IN ?

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Rhysodine beetles, like the vast majority of Geadephaga belong to the "Carabidae clausae", characterized by closed anterior coxal cavities. This is not a simple character, but involves major changes in several structures, including the development of a septum between left and right cavities, a vertical column posterior to the coxa, the elongation of the ventral posterior angle of the propleuron to form a postcoxal bar, and the modification of the tip of the bar and its reception into a pit in the column. It is correlated with elongation of the mesosternum, development of a constriction in it anterior to the middle coxae, loss of its median carina, and development of a peg-and-socket median articulation between mesosternum and metasternum. These common features strongly suggest that "Carabidae clausae" is a monophyletic group, and that Rhysodini, as well as Cicindelini, are members of it.

Most of the "Clausae" belong to the "Limbata Lobata", characterized by conjunct middle coxal cavities and a lobe of the metepimeron overlapping the base of the first abdominal sternum laterally. Rhysodines are excluded from this group, as are Cicindelini, Loricarini, Elaphrini and a few other tribes. Rhysodini have the most in common with Scaritini, including an elongate pedunculate mesothorax, front tibiae with processes extending beyond tarsal articulations, similarly shaped repugnatorial glands, similar disc-like, somewhat asymmetrical ventral articulation of the anterior coxae, and similar placement of some prothoracic muscles. These are enough to suggest a common ancestry of Scaritini and Rhysodini.

01-064

PHYLOGENETIC RELATIONSHIPS AMONG "BASAL GRADE" CARABID LINEAGES (COLEOPTERA: CARABIDAE)

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This report of research-in-progress on the phylogenetic relationships of basal grade carabid beetles centers on taxa of supertribe Nebriitae, including the Opisthiini, Notiophilini, Notiokasiini, Pelophilini, and Nebriini. Results of computer-assisted cladistic analyses of 370 adult external and internal genitalic morphological features suggest a sister group relationship between Notiophilini and Notiokasiini and between a clade comprising these two taxa and the Opisthiini. In turn, this three-taxon clade is the sister group of the Nebriini (excluding *Pelophila*), and tribe Pelophilini (genus *Pelophila*) is the sister group of a clade including all four other nebriite tribes. Relationships of this most inclusive clade to the Cicindini, Loricerini, and other basal-grade tribes are also discussed.

01-066

CLADISTIC RELATIONSHIPS OF THE TRIBE BROSCINI (COLEOPTERA)

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Broschini is a tribe of Carabidae with 255 species distributed in 35 genera. All Broschini are temperate species, occurring around the world both north and south of the tropics.

The tribe Broschini presents no apotypic state; to check its monophyly the cladistic analysis is developed with tribes representatives of the different subfamilies of Carabidae. The characters considered are those proposed for higher classification in Carabidae. Considered taxa correspond to 14 tribes included in different supertribes, and five Broschini genera. The family Trachypachidae was chosen as the outgroup of the whole family Carabidae, following Kavanaugh (1986) and Erwin (1985), who suggested that *Trachypachus* is the sister group of the remaining Carabidae. Character transformations are polarized accordingly to outgroup. The cladistic analysis yielded 16 cladograms. All of them show that the Stylifera (sensu Jeannel) conforms a monophyletic group, where Apotomini, Melaenini and the Broschini genera constitute an unresolved polytomy. The successive weighting procedure yielded three cladograms which show Broschini as a monophyletic (but polythetic) group, as the sister group of Melaenini plus Apotomini, and the relationships between the others tribes is similar to those proposed by Erwin (1985). Other groups considered to be related to Broschini, as Elaphrini, appears in this analysis among the most distantly related taxa in terms of nodal separation of Broschini. Goulet (1983) proposed a close relationship between the tribes Broschini and Elaphrini, since both share the sclerites X and Y in the internal sac. However this structure might be homologous to these found in Melaenini, Paussini (rod of Darlington, 1950), Omophronini (basal piece of Lindroth, 1961b), and the basal stylets of endophallus present in *Schizogenius* (Whitehead, 1972). If this structure is distributed in many tribes, it could be a plesiomorphic structure lost in different tribes. This result is congruent with Erwin's (1985) point of view, that the Elaphrini might be related to Migadopini, not with Broschini. He based his idea because Elaphrini has two larval claws, while Broschini has one.

A second cladistic analysis, using 75 characters, 82 Broschini taxa, and Melaenini and Apotomini as sister groups yielded one cladogram using successive weighting procedure. Four main generic groups are suggested as subtribes. The value of several characters are discussed with reference to Broschini classification.

01-065

CONFLICTS IN PHYLOGENETIC RELATIONSHIPS AND DISPERSAL HISTORY OF CARABITAE (COLEOPTERA, CARABIDAE).

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The tribes of the Carabidae are distributed in a characteristic manner. In the southern hemisphere the Ceroglossini (*Ceroglossus*) are restricted to southwestern South America, the Pamborini to southeastern Australia (*Pamborus*) and New Zealand (*Maori-pamborus*). The Cychrini and the genus *Carabus* s. l. are holarctic. Only *Calosoma* s. l. is distributed world wide.

The Carabidae quite clearly form a monophyletic group, but the relationships of their tribes are questionable: the tree established by Moore (1966) postulates a close relationship of Cychrini and Pamborini and the dispersal processes postulated by Erwin (1979) are based on it.

We analysed a set of morphological characters of adults and larvae which also gives some evidence to support alternative hypotheses concerning the southern tribes.

In addition, we sequenced different genes of the mitochondrial DNA of these taxa. These data were used for a re-analysis of the phylogenetic relationships of the Carabidae tribes as well as for an estimate of their divergence times. Based on these data, we present alternative interpretations of the dispersal processes.

Using the transition/transversion rate of base substitutions of the species under study and of other insects from the literature, the Carabidae split into the extant tribes in a period later than the pangaean, therefore, the spread of the tribes must have occurred independently.

01-067

A PRELIMINARY SURVEY OF THE CLASSIFICATION OF THE PSYDRINAE (COLEOPTERA: CARABIDAE)

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Psydriinae constitute a very heterogeneous assemblage, both in external and genitalic morphology. Psydriinae as a whole or one of their tribes are believed to represent a group very close to the stem-group from which one of the major branchings within Carabidae arose. The relationships of the tribes of Psydriinae are tracked using the cladistic method sensu Hennig. Except for Psydrini and Melisoderini the monophyly of the tribes can be demonstrated. The monophyly of Psydriinae as a whole is uncertain, because Psydrini are so primitive that even closer relationship of Patrobinae with the remaining 5 tribes of Psydriinae cannot be excluded. The latter form a monophyletic group that splits into the monophylum Tropidopterini-Melisoderini-Amblytelini, whereas the relations of Mecyclothoracini and Meonidini are still uncertain. It is controversial whether Tropidopterini and Melisoderini or Melisoderini and Amblytelini form a further monophylum. Psydrini are certainly the most primitive tribe while Amblytelini are most evolved. Amblytelini and several species groups of Mecyclothoracini have adopted an arboreal or semi-arboreal way of life, and they have also crossed the borders of the wet forests of antarctic origin. Psydriinae (Psydrini excluded!) may have originated in the former Gondwanaland and belong to the "younger antarctic element" in the faunas of Australia and South America. Due to their primitiveness and their occurrence in the Holarctic Psydrini may have originated considerably earlier.

01-068

THE ENIGMATIC TRIBE HELLUODINI: BIOGEOGRAPHY, BIOLOGY AND SYSTEMATICS (COLEOPTERA: CARABIDAE)

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The Helluodini form a small but distinct group that is primarily Oriental in distribution, but with southern extensions to New Guinea and tropical Australia. Little has been published concerning the biology of its members but species of the predominant genus *Pogonoglossus* Chaudoir are known to be subcortical predators. Larvae now attributed to this genus provide further evidence in support of separate tribal status for the group.

01-070

EVOLUTION AT THE EQUATOR: ARBOREAL AND ALTICOLOUS BEETLES AND THEIR TAXON PULSES (COLEOPTERA: CARABIDAE)

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ABSTRACT - The neotropical carabid genera *Agra* (Lebiini - Calleidina) and *Batesiana* (Bembidiini-Xystosomina) contain more than 2,100 species, the former with 2,000+ and the latter with ca. 100. One monophyletic lineage of each, the *arrowi* group of *Agra* and the *parainsularis* group of *Batesiana*, was selected for phylogenetic analysis to underpin a biogeographic study pertaining to the Amazon Basin and its mountainous southeastern and western flanks. Both groups arose and radiated from the basin, subsequently invading Central and/or Middle America and the Andean highlands, as well as the South Atlantic Forest. The fact that such major radiation occurred within the basin at the species level in two unrelated significant clades forces us to look for evolutionary driving forces that act relatively quickly and at a fine geographic resolution in order to account for the megadiversity found there today. What are the possibilities? Of those discussed in this paper, the one with the least study is the latest to be introduced - the long term cyclic impact of El Niño on both the forest and the populations of organisms using that forest. Will drought and subsequent fires resulting from hypothesized El Niño events be extensive enough to isolate forest patches, as previously suggested for the pleistocene forest refugia? Will these isolated forests exist long enough for vicariant speciation? How do populations of insects respond to drought ridden tropical forests even when these don't burn? What are the effects of shorter cycles on these same populations, for example, the 4 year El Niño cycle? Is there a connection between cyclic abundance highs and lows and dispersal that might lead to a founder effect across isolated forest patches? Does tropical lowland drought force taxa in higher elevations that act as refugia and a path of no return? These are but a few of the questions raised by newly aquired data and a bold new suggestion - massive Amazonian fires? Is the current activity of humans there *deja vu*?

01-069

PHYLOGENY AND ZOOGEOGRAPHY OF CALLEIDINA (COLEOPTERA: CARABIDAE: LEBIINI) : A PRELIMINARY SURVEY

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Calleidina are a large assemblage of genera and species of Carabidae Lebiini distributed in all continents, with their highest diversity in intertropical areas. Most of them are specialized arboreal beetles, tied to foliage in forest canopies. No recent revision of the group is available to make evident the phylogenetical relationships among taxa - many of which undescribed - from the different parts of the world. In the present contribution, preliminary results of a revision of the former genus *Calleida* Dejean, 1825 (*Callida* Auctorum, in the widest sense) are given. Particularly, two main questions have been posed at the present time: 1) If Calleidina, in the sense of the authors, are a monophyletic unit; and : 2) If the genera and subgenera into which *Calleida* has been split are monophyletic groups. On the base of a cladistic analysis of several characters, it is possible to establish the limits and the systematic position of Calleidina among other subtribes of Lebiini. Furthermore, it seems clear that some supraspecific taxa, proposed or re-defined by different authors, are polyphyletical or paraphyletical assemblages, needing a complete revision. Previous and new characters, and their polarity based on outgroup comparison (including Demetriina, Peliocypina, and Agrina), are discussed, in the light of possible transformation series and convergence of features in specialized arboreal Carabid beetles. Some distribution patterns are examined. Relationships among groups of species or subgenera from Africa, Madagascar and S.E.Asia, became clear. Less evident, but highly probabilistic, seem to be the affinities among some Afrotropical and Neotropical *Stenocallida* and *Calleida*, respectively. The origin of some lineages dispersed to Middle America and the West Indies have also been pointed out.

01-071

LABOULBENIALES (ASCOMYCETES) AND SYSTEMATICS OF THEIR CARABID HOSTS (COLEOPTERA: CARABIDAE)

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Laboulbeniales are parasitic fungi on living arthropods, mostly insects. They are strictly adapted to parasitic life, as reflected in their peculiar morphology, in the very limited damage caused to their hosts, and in their high host specificity. The latter feature, which is sometimes overemphasised, can, however, provide useful clues in understanding the evolution of their hosts, especially when the systematics of the parasites is reliable. For instance, the same *Dimeromyces* species parasitizes both Afro-Malgascian and Mediterranean Reicheina indicative of a close relationships. Moreover, historical geographical patterns of the different species-groups belonging to the *Duvalius* genus in the Mediterranean area has been successfully reconstructed by analysing their associated Laboulbeniales. Phylogenetic relationships amongst different groups can be drawn by matching hypotheses with data on parasites. *Mexisphodrus* is parasitized by the same Laboulbeniales of the Platynini (not by those of the Sphodrini); the Australian genus *Mecyclothorax* (presently included in the Psydrini) bears fungi similar to those of the Trechini; true relationships of several holarctic species of the "old" *Pterostichus* genus now placed within different tribes or subtribes are witnessed by the different species of associated *Laboulbenia*. Higher ranking is currently under investigation and is providing some interesting data. For instance, the tribe Agonicini seems to merit a place close to the Psydrini; Odacanthinae and Hexagoninae bear similar fungi. Laboulbeniales also seem to support the affinities between Paussinae (*Pseudozena*) and Brachinininae (*Pheropsophus*) as suggested by biochemical studies, somewhat in contrast with morphological data.

01-072

PHENOLIC DIVERSITY IN UNGALLED, SINGLE GALLED AND MULTIGALLED STEMS OF COTTON INDUCED BY THE COTTON STEM WEEVIL, *PEMPHERULUS AFFINIS* FST. (COLEOPTERA: CURCULIONIDAE)

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The cotton stem weevil, *Pempherulus affinis* Fst. was found to induce two types of stem galls viz. single and multiple stem galls on cotton, *Gossypium hirsutum* (Cultivars-LRA, SUVIN, MCU-5) and the weed host plant *Sida acuta*. The present study explains the quantitative changes in total phenols, OD phenols, tannins and gossypol among different host plants as well as different levels of infestation. Results revealed that the stems of cotton cultivar LRA and *Sida acuta* showed lesser levels of all the secondary plant metabolites, whereas between different levels of infestation, multigalled stems recorded maximum amounts of these allelochemicals. Individual phenols also showed a differential distribution among different host plants as well as different types of galls. In addition, pyrogallol was found to be the major phenol of single galled stem, whereas gentisic acid and gallic acids were the major phenolic acids recorded in multigalled stems.

01-074

PLANT-HERBIVORE INTERACTIONS AND ANTENNAL MORPHOLOGY STUDIES OF THE BLACKCURRANT LEAF MIDGE *DASINEURA TETENSI* (DIPTERA, CECIDOMYIIDAE). D.J. Crook¹, A.N.E. Birch², R. Brennan², J.A.T. Woodford², A.J. Mordue¹ (Luntz).

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The Blackcurrant Leaf Midge (*Dasineura tetensi*) in recent years has become a major pest of blackcurrant (*Ribes nigrum* L.) throughout the world. Larval feeding causes severe distortion of young leaves and affects plant fitness. Several sources of resistance to *D. tetensi* have been identified in different *Ribes* cultivars but little is known of the mechanisms. During this project the effects of cultivar type on the behaviour of *D. tetensi* will be investigated in both the laboratory and field and the plant-herbivore interactions of the cultivars and midge will be determined.

Investigations carried out using scanning and transmission electron microscopy on the antennae of *D. tetensi* have shown that males and females share the following five types of sensillum: sensilla chaetica, sensilla trichodea, sensilla basiconica, circumfila and terminal sensory pegs. The morphology of each sensillum type is discussed in detail.

Oviposition preference has been studied on resistant and susceptible cultivars in both the laboratory and field. Both linear-track and Pettersson olfactometers along with electroantennogram recordings (EAG's) are being used to establish behavioural and electrophysiological differences elicited in the adults when exposed to both resistant and susceptible *Ribes* cultivar leaf volatiles.

01-073

OPPOSITE EFFECTS OF PLANT STRESS ON DIFFERENT LIFE-STAGES OF A GALL-MAKING APHID

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The impact of plant stress on the performance and occurrence of gall-making aphids (Homoptera: Adelgidae) was investigated by estimating the size and density of *Sacchiphantes abietis* galls on experimentally stressed Norway spruce trees. Stands of spruce trees were treated so as to create a gradient of tree 'vitalities'. Hence, trees were exposed to different combinations of water and nutrient treatments and comprised a control and the following five treatments: drought, irrigation, ammonium sulphate addition, nitrogen-free fertilization and optimum fertilization with irrigation.

The density of galls was higher on drought-stressed trees than on all other treatments in the second year of the experiment. Possible mechanisms behind this positive 'all or nothing'-type of effect of drought on the aphids are discussed. The proportion of trees with galls did not differ among treatments.

Results on the size of galls and indirect measurements of the fecundity of stem-mothers suggested that stress influences these two parameters oppositely. The positive correlation between spruce growth and gall growth supports the Plant Vigour hypothesis, whereas the negative correlation between spruce growth and number of cells per gall (an indirect measure of stem-mother fecundity) supports the Plant Stress hypothesis. However, these relationships could only be discerned when gall parameters were correlated to the growth potential of the attacked shoot and not on the stand level.

01-075

THE SPERM ULTRASTRUCTURE OF DIPTERAN CECIDOMYIIDAE

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Spermatozoa in Cecidomyiidae lack acrosome and accessory tubules. In other respects and in particular with respect to the flagellar axoneme they display a unique diversity. In Porricondylineae the plesiomorphic genus *Diallactes* (Diallactini) shows an almost conventional 9+2 axoneme, while a 9+3 axoneme is shared by all the other tribes except for Winnertzini which exhibit a 9+0 pattern.

In Lestremiinae there are spermatozoa with a 9+0 axoneme (*Peromyia*, *Bryomyia*, *Heterogenella*), those with 10 (*Xylopriona*, *Polyardis* and *Micromya*) or 20 (*Anaretella*) microtubular doublets arranged in a circle, or also axonemes made of antiparallel spirals of doublets (*Lestremia*).

Cecidomyiinae have several supertribes that can be distinguished by their axonemal models. In Cecidomyiidi the sperm axoneme consists of single rows of doublets; in Asphondyliidi of double, parallel spirals of doublets; in Lasiopteridi of a 9+0 surrounded by singlet microtubules. *Contarinia* and related genera among Cecidomyiidi and *Rhizomyia* among Lasiopteridi have sperm axonemes with microtubular doublets devoid of dynein arms and housed in scalloped plasma membrane; it is suggested to place them in a separate group. Stomatosematidi is a paraphyletic assemblage with *Didactylomyia* and *Stomatosema* showing different axonemes.

01-076

A GENERIC REVISION OF THE LASIOPTERINI OF THE WORLD (DIPTERA: CECIDOMYIIDAE: LASIOPTERIDI)

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The 270 species of known Lasiopterini are distributed among fewer than 15 genera, all but *Lasioptera* restricted by plant host preference and defined by shared, derived characters. *Lasioptera*, containing more than half the species, remains an omnibus genus, although it includes some evidently monophyletic species groups that are restricted by region and host.

01-078

RECENT TAXONOMIC RESEARCH ON THE AFRICAN RICE GALL MIDGE, *ORSEOLIA ORYZIVORA* HARRIS & GAGNÉ

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Orseolia oryzivora Harris & Gagné has become a major pest of rice in West Africa during the past decade at a time when rice production has been intensified to meet local needs. A research project initiated by the West Africa Rice Development Association in collaboration with the CAB International Institutes of Biological Control (IIBC) and Entomology (IIE) has included taxonomic studies of *Orseolia* species on cultivated and wild rices and on other host plants. Results of these studies are reported and compared with results of earlier work by Gagné on the Asian rice gall midge, *Orseolia oryzae* (Wood-Mason), and other *Orseolia* species in the Oriental region.

01-077

INDUCED NEAR-IMMUNE RESISTANCE IN WILLOW PROMOTES RAPID LOCAL ADAPTATION IN A GALL MIDGE.

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Certain genotypes of *Salix viminalis* display an immediate and near-immune resistance in response gall initiation attempts by the leaf-roller gall midge, *Dasineura marginemtorquens*. In monoclonal plantations of resistant willows midge population numbers are initially very low but increase dramatically within a few years. We suggest that frequencies of virulent midges are low initially and increase in response to strong selection by the resistant willow. Several aspects of *D. marginemtorquens* life history fill requirements conducive for rapid host adaptation: it is monophagous and has 3-4 generations each season, adults are short lived, mating and oviposition occur on the plant from which larvae developed, mating is non random since midges from resistant plants have a longer developmental time, they seldom encounter mates from susceptible plants.

01-079

FIRST RESULTS OF THE REVISION OF THE LESTREMIINAE FROM THE HOLARCTIC REGION (DIPTERA: CECIDOMYIIDAE)

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By using the method of comparative morphology of imaginal characters the lestremiin species described from the Holarctic Region are revised in their entirety for the first time. Since Meigen, in 1818, 462 species in 65 genera were described belonging to the Lestremiinae.

So far as accessible the type material formed the basis for this study, completed by further material from all main lestremiin collectiones including about 3000 specimens of 120 species collected by the author in recent years. Further refinement in investigating morphological characters (particularly concerning the male genitalia and the arrangement of bristles) and another valuation of variability and the occurrence of aberrations (as a result of technical manipulations) proved to be keys for numerous changes at the level of species. Around 50% of the Nearctic species occur in the Palaearctic, too, and the large majority of species inhabits a wide range within the Palaearctic Region (causing double descriptions in former and recent times). Including the description of about 35 species new to science (mainly belonging to *Apriomus* and *Peromyia*) we may assume 280 known valid species in 40 genera of Lestremiinae. Contrasting with these changes, the system of taxa at supraspecific level (genera and tribes) seems to be more constant in relation to the existing ideas, also considering our poor knowledge from other faunas. A phylogenetic classification of the Lestremiinae is proposed at the final stage of the revision.

01-080

NOTES ON THE TAXONOMY AND DISTRIBUTION OF THE
GALL-MIDGES OF THE GENUS *KARSCHOMYIA* FELT
(DIPTERA, CECIDOMYIIDAE)

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The Revision of the materials on the genus *Karschomyia* Felt from Russia discovered some new objectives. Mamaev (1969) synonymized the genera *Karschomyia* Felt, 1911 and *Plesiobremia* Kieffer, 1912. Gagne (1972) reported that the genus *Hiastatus* Marikovsky, 1956 is the junior synonym of the genus *Karschomyia* Felt. In our opinion there is one distinct character - the absence of the lobe on the gonostylus in *Hiastatus* species - which must be considered at least as subgeneric. So I separate 2 subgenera: *Karschomyia* Felt (= *Plesiobremia* Kieffer) and *Hiastatus* Marikovskij. It is possible that in future the genus *Hiastatus* Marikovskij will be restored as valid genus. The revision besides several new species increased the number of gall-midges with Holarctic distribution. They are *K.viburni* Felt, 1907 (= *K.aceri* Mamaev, 1960, syn.nov.), *K.caulicola* Cog., 1895 (= *K.concinna* Marik., 1956, syn.nov.) and *K.hemispherica* Kov. et Mam., 1966 (= *K.insolita* Gagne, 1973, syn.nov.)

01-082

THE SPECIES CONCEPTION IN FREE DEVELOPING
GALL MIDGES

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The identification of free developing gall midges mainly depends upon morphological characters of male postabdomen, investigated under light microscope. It is the first, the simplest stage of taxonomic investigation. When investigators cannot distinguish similar species they usually list them as synonyms. In the future these synonyms are not taken into consideration. The second stage of taxonomic investigation preferably evaluates all morphological and biological characters and also apply conceptions of vicarious specie, sibling species etc. A number of free developing gall midges are very similar as males, but well distinguishable as females and larvae. Externally very similar species of *Miastor* can be distinguished according to their life-cycle. Indistinguishable males of similar species may be distinguished according to different sperm ultrastructure.

Externally very similar species from different geographic isolated provinces belong to different so-called vicarious species. The origin of these species is the result of genetic isolation of populations. Most of similar Palaearctic and Nearctic gall midges seem to be vicarious species.

The species conception of free developing gall midges has to be co-ordinated with general species conception in biology, including morphological (all stages of development), ultramorphological, biological, zoogeographic and genetic criterions.

01-081

A PHYLOGENETIC ANALYSIS OF HIGHER-LEVEL GALL
WASP RELATIONSHIPS

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A phylogenetic analysis is made based on morphological characters of adults. The study comprises thirtyseven cynipid species from thirtyone genera representing all six currently acknowledged tribes. From the paraphyletic, and thus problematic, tribe Aylacini nineteen of the twentyone known genera are included. This is the most comprehensive analysis of gall wasp relationships conducted thus far. The results support the monophyly of Synergini and Cynipini as well as that of a larger group consisting of the cynipids making galls on woody rosids, viz. Eschatocerini, Rhoditini, Pediaspidini and Cynipini. There is also strong support for placing *Himalocynips*, suggested to belong to a separate subfamily, in Pediaspidini together with *Pediaspis*. Furthermore, the results suggest a possible division of Cynipidae in three monophyletic groups. One group consisting of Synergini and four genera from Aylacini, a second (the sistergroup of the first) of only Aylacini genera and a third of the remaining six genera together with the aforementioned group of gallmakers on woody rosids. Taxonomic implications of the results are discussed.

01-083

GALL SITE PREFERENCE AND INTRASPECIFIC
COMPETITION OF *NEOTHORACAPHIS YANONIS*
(HOMOPTERA: APHIDIDAE)

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Some recent studies on galling aphids have put the preference-performance hypothesis in doubt. Our study of a gall aphid *Neothoracaphis yanonis* demonstrated that the stem mothers produce galls more abundantly on the leaves at lower positions on a shoot, and on the basal intermediate part of a leaf blade. On this leaf position and leaf part, aphids produced larger galls, containing more abundant alates. This result suggests that the aphid have evolved to synchronize with the opening of leaves at lower positions, but the leaf part was preferentially chosen. Thus, our observations support the preference-performance hypothesis which assumes that aphid selects the best habitat.

As gall density increased, gall size declined, the percentage of indehiscent galls increased, and the relative abundance of upper-type galls increased. The density effect resulted in promoting the dispersal of stem mothers and reducing the fitness of aphids which located on less favorable sites.

01-084

FACTORS INFLUENCING LOCAL DIFFERENCES IN THE POPULATION DENSITY OF THE CRYPTOMERIA BARK MIDGE, *RESSELIELLA ODAI* (I.) (DIPTERA:CECIDOMYIIDAE)

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The cryptomeria bark midge, *Resseliella odai*, is a pest of the Japanese cedar, *Cryptomeria japonica*, an important tree species for silviculture in Japan. The relationship between stand situation and midge density was analyzed. Altitude was the most important factor. Midge density increased slightly at elevations below ca. 800m in altitude, and densities were remarkably high in stands above this altitude. The increment of humidity and the decrement of temperature with elevation may partially explain this change, but the changes in these meteorological factors were linear, in contrast to the change in midge densities. Severely attacked trees in high elevation stands showed unusual tree trunk form because bark was almost completely removed by the bark midge and fungi. This may make further attack by the midge easier, and may explain the high midge density at high elevations. To test this hypothesis, two experiments were done.

01-086

PHYLOGENETIC PATTERNS IN THE EVOLUTION OF THE GALL WASP - HOST PLANT ASSOCIATION (HYM.: CYNIPIDAE)

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The early evolution of the gall wasp-host plant association was reconstructed by mapping biological data on recent hypotheses of higher-level cynipid and cynipoid relationships. The results indicate that gall wasps evolved from parasitoids of insect larvae living inside plant tissue, possibly inside seed capsules of *Papaver*. The gall was originally manifested externally solely by a simple but distinct swelling of surrounding plant tissue. The familiar, complex and species-specific galls on oaks and roses evolved later in a single, evolutionarily successful cynipid lineage galling woody rosids.

Most lineages of gall wasps remained associated with herbaceous hosts; woody hosts were apparently colonized only three times during the evolution of cynipids. The herbaceous hosts primarily belong to a narrow spectrum of genera within the families Asteraceae, Papaveraceae and Lamiaceae. The history of the association is characterized by periods of constraint interrupted by major shifts between apparently unrelated host plants. There are striking parallelisms in these host plant shifts; alternative hypotheses to explain this pattern are discussed.

The inquiline apparently originated from gall wasps inducing galls on *Potentilla*. When one lineage of these gall wasps shifted host plant to *Rubus* bushes, which were also galled by other, closely related cynipids, they lost their gall-inducing capacity and became inquiline.

01-085

THE GALL MIDGE *LASIOPTERA ARUNDINIS* HAS EVOLVED STRUCTURAL AND BEHAVIORAL FEATURES TO DISSEMINATE ITS FUNGAL SYMBIONT

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Lasioptera arundinis attacks the common reed stem. With the help of its associated fungus, the first instar larva penetrates into the stem and gains access to the vascular tissues of the host plant. The larva feeds both on the reed stem and on the fungal hyphae. The imago and the first larval stage have adapted structures to collect and to carry the fungus. The imago has evolved a specific preovipositional behavior which allows fungal spore collection and dissemination. Spores and small fragments of hyphae, collected along the upper internodes of the reed stem, are harvested in two pockets localized at the terminal part of the ovipositor. During oviposition, a canal leads the spores to the oviduct. Eggs and fungal spores are deposited together on the reed stem. These very specialized structures will be described.

01-087

SYSTEMIC ANATOMY OBSERVATIONS ON CECIDOMYIIDAE (DIPTERA, NEMATOCERA)

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A preliminary study on the gross anatomy of larval central nervous system, larval and imaginal digestive systems, and adult (both female and male) reproductive systems has been carried out with few gall midge species but looking forward to a more comprehensive study in the Cecidomyiidae family. The larval central nervous system has been confirmed to be formed by a relatively big cerebrum, located in the prothorax, a well developed suboesophageal ganglion, and a relatively short ventral nerve cord with 10 ganglia (last ganglion elongate and apparently compound). The alimentary canal has been investigated in *Mayetiola destructor* Say adults, in *Mycodiplosis tremulae* Kieff. larvae, and both in larvae and adults of *Monarthropalpus buxi* Lab. and *Aphidoletes aphidimyza* Rond. where it shows strong similarity between larval and imaginal mesenteron (always posteriorly blind) whereas both stomodaeum and proctodaeum undergo important modifications during metamorphosis. The reproductive system has been studied in *M. destructor*, *A. aphidimyza* and *M. buxi*, among which it shows a very similar structure (both in females and males). Possible physiological, behavioural, or ecological interpretations are discussed.

01-088

ROLE OF LARVAL GREGARIOUSNESS OF THE PINE NEEDLE GALL MIDGE, *THECODIPLOSIS JAPONENSIS* UCHIDA ET INOUE (DIPTERA: CECIDOMYIIDAE), IN ITS REPRODUCTIVE SUCCESS AND POPULATION CHANGES.

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The larval gregariousness of the pine needle gall midge, *Thecodiplosis japonensis* Uchida et Inoue (Diptera: Cecidomyiidae), did not cause the death of larvae in the gall but decreased the size of larvae. The decline in the larval size did not cause a simple decline in the number of eggs deposited per female. Females that emerged from medium size larvae deposited many more eggs than those from large and small larvae. The number of emerged adults per gall was expected to increase with the number of larvae per gall. The calculated number of eggs deposited per gall increased with an increase in the number of larvae per gall from one to five, but not when the number of larvae per gall was more than six. The oviposition pattern, depositing eight eggs on average per pair of needles, and the larval gregarious in galls may offer the midge the maximum reproductive success. On the other hand, when the population is in an outbreak phase, larval gregariousness may cause a drastic decline in the population density.

01-090

CUTICULAR STRUCTURES ON THE HEAD OF THE MATURE LARVA OF CECIDOMYIIDAE (DIPTERA), ADAPTIVE AND SYSTEMATIC ASPECTS

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The external structure of the head capsule of last instar larva of 23 species of gall midges representing different taxonomic groups and varying larval habits was examined using Scanning Electron Microscopy (SEM). Some structural characters, as for example the length of the antenna, were found to be adaptations to the life in the gall, or to a predacious or sporo-mycophagous life. The examinations also demonstrate synapomorphies for tribes, sub tribes, and genera. Evolution appears to have occurred mainly towards reductions of external parts of the sensilla.

01-089

TAXONOMY OF CERTAIN PALEARCTIC *DASINEURA* AND *JAAPIELLA* SPECIES (OLIGOTROPHINI, CECIDOMYIIDAE) DEVELOPING IN HEADS OF ASTERACEAE

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Many of the gall midges referred to here are characterised by a pronounced morphological conformity. However, not least on the basis of morphometric analyses a good understanding of the taxonomical relationships was obtained. The measurement values were corrected for allometric deviations, according to a method representing a further development of a procedure previously tested for taxonomical purposes in gall midges. Oligotrophine specimens originating from larvae collected in heads of plants distributed among eight genera of Asteraceae were taxonomically studied. No apparent galls are produced by these midges. Adults, pupae, and larvae were analysed. Several *Jaapiella*, and two *Dasineura* species are represented. Most species are new to science. Host plant relationships of the species are discussed.

01-091

RECOLONIZATION OF THE KRAKATAU ISLANDS, INDONESIA BY GALLING INSECTS AND MITES

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The Krakatau Islands are situated in the Sunda Strait between Java and Sumatra, Indonesia and consist of 4 islets, namely Rakata, Panjang, Sertung, and Anak Krakatau. As is well known, the former three islets were almost certainly completely sterilized by the world-famous large eruption in 1883, and the last islet emerged in 1930s by submarine volcanic activities. We visited the islands and the vicinities 16 times from 1981 to 1994 to study the succession of plants and their associated insects and mites, particularly those responsible for galls on various plant species. The galler's fauna of the Krakataus was similar to that of Java rather than to Sumatra and showed a distinct faunistic disharmony. Species number was still increasing, and turnover events were recognized during the course of plant succession. These results were compared with those for other herbivorous and predacious insects.

01-092

PHYLOGENETICS OF STREBLIDAE (DIPTERA) WITH A PRELIMINARY ASSESSMENT OF THEIR COEVOLUTION WITH BATS (CHIROPTERA).

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The primary goal of this research program is to advance understanding of the ecology and genetics of host-ectoparasite interactions. Here we outline a program of morphological and biochemical studies of batflies (Diptera: Streblidae) that is based on the alcohol collection at the Field Museum that is unparalleled in size, diverse in taxonomy and provenience, and thoroughly documented as to host and other faunal associates. The collection includes most valid species of streblids, as well as numerous undescribed forms. A total of 16,293 vials of batflies have been curated, labeled and organized into a fully relational database. The database is intended to serve at least three functions: (1) to rapidly identify and locate batfly specimens needed in either morphological or molecular analyses; (2) to process, label, and install incoming material; and (3) to serve as an enlarging cross-tabulation of parasite occurrences (and co-occurrences) on bats across the Neotropics. This latter feature of the database will be useful in identifying and framing coevolutionary questions.

01-094

MOLECULAR SYSTEMATICS AND POPULATION GENETICS IN POLYNESIAN BLACK FLIES: PRELIMINARY MITOCHONDRIAL DNA DATA.

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Hypothesis 1: Testing the monophyly of the subgenus *Inseliellum*.

We sequenced 340 base pairs (about 45%) of the mitochondrial DNA (mtDNA) *COII* gene of three species of Polynesian black flies in the subgenus *Inseliellum*. We chose *Simulium oviceps*, *S. cataractarum* and *S. tahitiense* because they are in distinctive clades in a phylogenetic hypothesis based on morphological characters, and they are morphologically diverged, at least in the larval stage. Samples were collected from 2 different river systems in Tahiti-iti and 3 in Tahiti. We found a low level of intraspecific variation (less than 5%). The three consensus sequences differed by 27 base pairs or approximately 8% divergence. Divergence at this level will likely provide sufficient informative characters for reconstructing the subgenus *Inseliellum* when all available species are used.

Hypothesis 2: Habitat specialist species display restricted intraspecific gene flow while generalist species have greater gene flow.

We chose three species pairs that are distributed only on the islands of Moorea and Tahiti. One of each pair is a specialist, found exclusively on cascade faces, while the second is a generalist, found in various types of streams. *Simulium cataractarum* and *S. oviceps*, the first species pair examined, display differences in restriction fragment lengths for mtDNA genes *ND4* and *ND5*.

01-093

A PHYLOGENETIC INTERPRETATION OF PALAEARCTIC SPECIES OF *HILARA* AND THE TRIBE HILARINI (DIPTERA: EMPIDIDAE)

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A first attempt of cladistic analysis of relationships between major groups of Palaearctic *Hilara* and for their natural classification.

The archaic lineage with a full set of plesiomorphic characters that belong to the ground plan of the genus *Hilara* is apparently represented by species classified in the *Hilara flavipes*-group. This complex of species is thought to be closest to the ancestral forms of the tribe Hilarini. On the contrary, the species of the *Hilara maura*-complex are considered to be the most specialized forms within the Palaearctic *Hilara*, characterized by the apomorphic loss of sensory bristles on body and legs.

The evolution of epigamic behaviour from the organized male synorchestia in biotopes distant from waters (in holoptic males of the *Hilara flavipes*-group) to unorganized, large, obviously emergence zonal swarms above water of the *Hilara maura*-complex (both sexes dichoptic), clearly follows the presumed phylogeny of *Hilara*.

Cladograms are presented showing the relationship between major groups of Palaearctic *Hilara*, and the monophyly of Hilarini is supported by ground plan characters.

01-095

THE HIGHER-LEVEL PHYLOGENY OF EREMONEURA (DIPTERA: BRACHYCERA)

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Phylogenetic relationships of the major lineages of Eremoneura (Empidoidea + Cyclorrhapha) are examined. The monophyly of the Empidoidea and its sister group relationship with Cyclorrhapha is reconfirmed, despite the discovery of postgonite precursors in the ground plan of the male genitalia of Empidoidea.

The relationships of the major subgroups of Empidoidea are re-evaluated. The polyphyletic subfamily "Oreogetoninae" is broken into several groups and the relationships of the remaining subfamilies are presented in light of these component subgroups. Based on characters of the male and female genitalia, the Brachystomatinae is considered the sister group of the Trichopezinae + Ceratomerinae lineage and this clade is hypothesized to be the sister group of the "Microphorinae" + Dolichopodidae lineage. Together these five subfamilies are tentatively considered the sister group of all remaining Empidoidea based on the apomorphic loss of acanthopores in the latter lineage. This latter assemblage contains several well-defined component lineages including the Clinocerinae, the Empidinae + Hemerodromiinae, the "Ocydromiinae" + Tachydromiinae + Hybotinae clade and its sister group the Atelestinae, and various genera and generic groups previously placed in the "Oreogetoninae" or a few other subfamilies.

The relationships of the basal lineages of the Cyclorrhapha are also examined. Opetiidae and Platypetidae are considered to be the basal lineages in the infraorder, whereas the Lonchopteridae is hypothesized to be the sister group of the Phoroidea (Ironomyiidae + Sciadoceridae + Phoridae). The Syrphoidea (Syrphidae + Pipunculidae) is considered the sister group of the Schizophora. Relationships within the diverse schizophoran lineage remain largely unresolved.

01-096

USING DROSOPHILA AS A PARADIGM IN SYSTEMATICS; AT THE INTERFACE OF DEVELOPMENT AND EVOLUTION.
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Drosophila melanogaster is one of the premier model organisms of the twentieth century. Its use in genetics, evolution, development and molecular biology has greatly advanced our understanding of these fields. More recent work on many of the closely related taxa to *D. melanogaster* in the dipteran family Drosophilidae has expanded systematic vistas too. This talk will discuss the family Drosophilidae as a paradigm for enhancing our understanding of systematic analyses and principles. The first topic for discussion in the talk will concern an examination of how molecular analyses have enhanced systematic studies of the group. In particular, the role of molecular sequence characters and their combination with morphological characters will be discussed. The second part of the talk will focus on the relatively new field of comparative molecular biology and developmental biology. The use of modern molecular techniques such as in situ hybridization and dissection of developmental processes can add to the number and types of characters that can be used in Drosophilidae systematics.

01-098

PHYLOGENETIC ANALYSIS OF CHARACTERS OF THE FEMALE REPRODUCTIVE TRACT OF ACALYPTRATE SCHIZOPHORA (DIPTERA)

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"The phylogenetic classification of the Acalyptratae is probably the most difficult chapter in the systematics of Diptera (McAlpine 1989)." It has been repeatedly suggested that for further resolution the morphology of the internal female reproductive tract needs to be investigated comparatively (Hennig 1973, McAlpine 1989).

An extensive collection of data on a number of characters of the internal female reproductive tract is presented and cladistically analyzed. Such characters are the number and form of the spermathecae, the occurrence and organization of a ventral receptacle, and the occurrence of other evaginations or sclerotized structures in the vagina. All 64 (McAlpine 1989) families of acalyptrate Schizophora are included. The plesiomorphic spermathecal number of Schizophora is thought to be 3. However, in the prevailing majority of acalyptrate families, genera with 2 spermathecae occur. In 34 families 2 is the only known condition. It can be assumed that 2 spermathecae are plesiomorphic not only for the Ephydroidea but also for Opomyzoidea and Carnoidea. The presence of a ventral receptacle is established in 56 families, but it is probably present in all of them. Multi-chambered forms occur in at least 21 families. Moreover in 8 families ring-shaped sclerotizations are found in the ventral wall of the vagina. While these characters could help to resolve phylogenetic questions, such as the position of Cremifanniidae, Neminidae, and Acartophthalmidae, the occurrence of additional evaginations of the vagina does not seem informative above the family level.

These data are a valuable resource to other researchers, providing information about conditions within families as well as comparative information about outgroups.

01-097

MOLECULAR PHYLOGENETIC ANALYSIS OF DIPTERAN PHYLOGENY BASED ON 28S RIBOSOMAL DNA SEQUENCES

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The long standing issue of Dipteran phylogeny has remained very controversial. More recently, the results of a cladistic analysis by Wood and Borkent (1989) primarily using larval character states suggested the polyphyly of two of the major subgroups, the Bibionomorpha sensu lato and the Tipulomorpha sensu lato, as they were proposed by Hennig (1981) mainly based on adult character states.

We have approached this problem by means of molecular phylogenetic analysis. We have sequenced DNA fragments encompassing the D1 and D7 region of the large ribosomal subunit RNA (28S rRNA) from a number of Dipteran species and representatives of closely related holometabolous insect orders. In the molecular phylogenetic reconstruction, monophyly is highly supported in the case of five major Dipteran subgroups, namely Brachycera, Tipulomorpha sensu stricto, Bibionomorpha sensu lato, Culicomorpha and Psychodomorpha. Accordingly, the controversial subgroup Bibionomorpha includes the families Sciaridae, Mycetophilidae, Cecidomyiidae, Scatopsidae and Anisopodidae as proposed by Hennig (1981), while the phylogenetic position of the Trichoceridae remains uncertain.

However, the phylogenetic relationships between these major subdivisions could not be robustly resolved with the present amount of sequence data. Still, morphological and additional molecular sequence data consistently support a sistergroup relationship between Bibionomorpha and Brachycera.

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01-099

THE IMPORTANCE OF FOSSIL MATERIALS TO INVESTIGATIONS ON HIGHER-LEVEL PHYLOGENY OF THE DIPTERA

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The oldest fossil Diptera from Lower/Middle Triassic of France (Krzemiński et al. 1994), from Middle/Upper Triassic of Asia (Shcherbakov et al., 1995) and from Lower Jurassic of Germany (Ansorge, 1994) allow to verify some concepts concerning relations between large lineages of Diptera and to explain homology of some veins (especially of anal field) of Diptera and Mecoptera.

1. *Grauvogelia arzwilleriana* from France, the oldest fossil fly known, has venation showing a combination of characters observed in different dipteran groups and does not fit any previous groundplan of dipteran wing.

2. One specimen from Lower/Middle Triassic of France (in prep.), and Diptera from Triassic of Asia: *Gonomusca renyxa*, *Psychotipa predicta*, *P. depicta*, *Mabelysia charlesi* show gradual elongation of A2 in Polyneura.

3. Lower Jurassic species, *Nannotanyderus krzemiński* (Tanyderidae) and *Tanypteryx connexa* (Psychodidae) of venation close to tanyderid type (with d cell) prove that Psychodidae had branched off the Tanyderidae. Both families represent the most primitive dipteran lineage, the Diarchineura (Krzemiński, 1992).

01-100

THE WING ULTRASTRUCTURE OF BIBIONOMORPHA AND ITS EVOLUTIONARY SIGNIFICANCE

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The comparative study of wing ultrastructure was fulfilled. The representatives of several, previously considered to be related, families of Bibionomorpha: Bibionidae, Pachyneuridae, Pleciidae, Cramptonomyiidae, Hesperinidae, Mycetobiidae, Canthyloscelidae and Axymyiidae were chosen for the investigation. The results demonstrated the next objectives: the structure and pattern of macro- and microtrichia of wing have certain significance for the specifying of relationships. Sharp difference between *Mycetobia-Hyperoscelis-Protaxomyia* group and the rest of bibionoid flies testify to more fractional division of Nematocera into infraorders.

01-102

ADULT CHARACTERS VERSUS LARVAL CHARACTERS IN PHYLOGENETIC STUDIES OF THE SCIOMYZOIDEA (CYCLORRHAPHA)

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The Sciomyzoidea are a problematic higher taxon within the acalyptate flies for several reasons: (1) Its monophyly is not supported by convincing characters. (2) Its composition is controversial with only Griffiths including the Cremifanniidae and Megamerinidae. (3) The limits of the largest family, the Sciomyzidae, are uncertain. The Salticellinae and Sciomyzinae form an undisputed monophyletic core group (Sciomyzidae sensu strictu), but its relationships to the Huttoninac, Phaeomyiinae and Helosciomyzinae are unclear. (4) The relationships among the constituent families of the Sciomyzoidea are unresolved. Hennig only recognizes one monophyletic group (Ropalomeridae + Sepsidae). J. McAlpine argues for an additional one consisting of the Sciomyzidae sensu lato and the Dryomyzidae and a basal position of the Coelopidae. D. McAlpine argues for a sister-group relationship between the Coelopidae and Dryomyzidae.

Larval characters have been largely ignored although they proved useful for a discussion of the relationships within the Sciomyzidae s.l. (Knutson et al.) and Sepsidae. Based on the tubercles of the last segment, the Sciomyzoidea can be divided into two groups. Several pairs of tubercles are found in the Dryomyzidae, Sciomyzidae s.s., and Helosciomyzidae. Unknown from the closely related families in the Lauxanoidea, the tubercles are putatively homologous and consistent with J. McAlpine's monophyletic group consisting of the Dryomyzidae and Sciomyzidae s.l.. However, larval characters do not necessarily clarify the phylogenetic discussion. For example, several characters point towards a sister-group relationship between the Sepsidae and Coelopidae questioning the one longstanding monophyletic group within the Sciomyzoidea that is supported by adult characters.

Despite the confusion about the composition and relationships, the Sciomyzoidea, rich in unusual natural history, deserve more attention. Some larvae are predators or parasites of molluscs (Sciomyzidae s.s.), barnacles (*Oedoparena*, Dryomyzidae), millipeds (*Pelidnoptera*, Phaeomyiinae), and possibly ant larvae (Helosciomyzidae). But whether the parasitic or predatory modes of life evolved independently will remain unknown until the phylogenetic relationships have been reconstructed.

01-101

PHYLOGENETIC UTILITY OF NUCLEAR GENES IN THE STUDY OF NEMATOCERA RELATIONSHIPS

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The suborder Nematocera is an important group of flies within Diptera as most of the disease vectors such as mosquitoes, sand flies, biting midges and black flies are included within this group. Recent phylogenetic studies have generated opposing views regarding the relationships within Nematocera. The major points of discrepancy are: (1) Wood and Borkent placed Tipulidae as the sister group to rest of the Diptera and moved Trichoceridae to the Psychodomorpha clade. Oosterbroek and Courtney placed Tipulidae+Trichoceridae clade as the sister group to Anisopodidae+Brachycera while Ptychopteromorpha+Culicomorpha was placed as the sister group to all other Diptera. (2) Aximyidae was removed from the Bibionomorpha clade and placed in a separate clade by Wood and Borkent, whereas in the latter study Aximyidae was the basal taxon in the Bibionomorpha clade. Oosterbroek and Courtney attribute the difficulties in interpreting relationships within Nematocera to the vast diversity of morphological characters, discrepancy between adult and larval characters, homoplasy, and lack of understanding of all character systems. Therefore, in order to resolve the relationships within Nematocera it is essential to look for new character data sets such as molecular characters.

Since character consistency appears to be a major factor in resolving the phylogenetic relationships of this group, we have undertaken an analysis of the phylogenetic signal present in several nuclear gene systems for this taxonomic level, by using representatives of ten families of Diptera. These gene systems are phosphoenolpyruvate carboxykinase, wingless, enolase, dopa decarboxylase and 18S rRNA. We present two ways of assessing the utility of these gene systems. First, we will compare the degree of character consistency and retention in each data set, and the degree of sequence divergence in pairwise comparisons. Second, we will place the character information in a total evidence (simultaneous analysis) framework and examine these characters on the basis of character congruence. We will incorporate morphological data also into the total evidence framework, and test congruence of the various character sets with each other and the total evidence hypothesis using the Incongruence Length Difference of Farris and Mckevich (ILD). A consideration of a combination of the ILD studies, and the saturation and consistency studies will give a more complete picture of the utility of these character sets.

01-103

CRANEFLY PHYLOGENY: A CLADISTIC ASSESSMENT OF MORPHOLOGICAL CHARACTERS OF THE ADULT HEAD AND THORAX (DIPTERA: TIPULOIDEA)

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The relationships of the major tipuloid lineages are cladistically analyzed using the parsimony program NONA. More than seventy characters, all pertaining to the skeleton and musculature of the head and thorax of the adults, have been scored for 23 exemplar species of crane flies belonging to the traditional families Tipulidae s.s. (2), Cylandrotomidae (1) and Limoniidae (20). The analysis also includes species of Mecoptera and Diptera (Trichoceridae, Tanyderidae, Ptychopteridae, Tabanidae) as outgroup taxa.

The results of the analysis are presented and used to assess the monophyly and cladistic affinities of the Tipuloidea. Further, my results are compared with previous, manual attempts on revealing the phylogeny of crane flies, notably those of Stary (1992) who based his analysis on a small selection of adult characters, and Oosterbroek & Theowald (1991) who used a large number of larval and pupal characters.

Finally, the prospects of arriving at a satisfactory family-group classification of the crane flies are outlined.

01-104

PHYLOGENY AND GEOGRAPHICAL DISTRIBUTION OF *ACHALCUS* (DOLICHOPODIDAE, DIPTERA)

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The genus *Achalcus* Loew, 1857 has a peculiar geographical distribution which is restricted to the temperate climatic zones of the world. The Holarctic (2 species) and Palearctic Regions (5 sp.) are considered to be rather poor in species, whereas a considerably richer fauna occurs in the Neotropical (Southern Chile: 3 sp. + at least 7 undescribed sp.) and Australasian Regions (Australia: 20-25 undescribed sp., New Zealand: 8 sp.).

Most Palearctic species seem to prefer marshland habitats and reedmarshes (*Phragmites australis*) in particular, except for *A. melanotrichus*, which is entirely confined to tree rotholes in which its larvae seem to breed. My recent systematic revision of the Palearctic *Achalcus* fauna not only added 4 new species to science but also revealed a considerable morphological difference between the arboreal *A. melanotrichus* and its congeners. This study also demonstrated that *A. melanotrichus* showed the most primitive features. As southern Chilean species are known almost exclusively from *Notophagus* woodlands and most Australian species are considered to be arboreal too, the phylogenetic relationship between the Holarctic, Neotropic and Australasian species is investigated, with special emphasis on the phylogenetic position of *A. melanotrichus*.

Data on their phylogeny and ecology are analyzed in an attempt to explain their present disrupted geographical distribution by reconstruction of the dispersal routes of ancestral species.

01-106

PHYLOGENY AND EVOLUTION OF THE GENUS *RHAGOLETIS* (DIPTERA: TEPHRITIDAE)

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The genus *Rhagoletis* (Diptera: Tephritidae) is composed of more than 60 described species distributed throughout Eurasia and the New World. Several *Rhagoletis* species are agricultural pests, infesting fruit of apple, cherry, blueberry, currants and walnuts. Because many *Rhagoletis* species are thought to have arisen in sympatry by shifting and subsequently adapting to new host plants, *Rhagoletis* is also a model system for studying modes and mechanisms of speciation. To study the evolution of host preference and establish the historical pattern of host shifts and speciation in *Rhagoletis*, we are using molecular and morphological characters to resolve phylogenetic relationships both within *Rhagoletis*, and between *Rhagoletis* and its Trypetine relatives. Interestingly, within two taxonomically-defined *Rhagoletis* species groups, morphological and molecular characters give conflicting phylogenies. *R. pomonella* and *R. cornivora*, and *R. cerasi* and *R. berberides*, represent two pairs of sibling species that are essentially indistinguishable using morphological characters. However, genetic distances based on both allozyme and mitochondrial DNA data suggest deep genetic divergence within each species pair. Possible explanations for this difference in morphological and genetic divergence (lineage sorting, selection, etc.) will be examined. Analyses of morphology, allozymes and mitochondrial DNA support the idea that *Rhagoletis* species in North American species groups form a monophyletic group. However, *Rhagoletis* monophyly breaks down when species in North American, Palearctic and Neotropical species groups are considered together. Previous ideas on the evolutionary origins of the North American *Rhagoletis* will be examined in the context of our current data, as will the apparently complex relationships between organisms currently classified as *Rhagoletis* and flies in other Trypetine genera (e.g. *Zonosemata*, *Oedicarena*, *Rhagoletotrypeta* and *Carpomya*).

01-105

THE PHYLOGENETIC RELATIONSHIPS OF THE CALLIPHORIDAE AND MYSTACINOBIIDAE (DIPTERA, OESTROIDEA)

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23 monophyletic taxa within the Oestroidea and two outgroup taxa (Muscidae and Anthomyiidae) were scored for 45 adult and larval characters and subjected to phylogenetic analyses searching for trees of maximum total fit (using Pee-Wee) or minimum length (using NONA and PAUP). The former gave 9 different trees for possible values of the concavity constant, all differing from the two minimal length trees. The trees obtained from maximum-fit searches are preferred since derived from a method that lowers the weight of characters showing within-terminal as well as within-tree homoplasy. The family "Calliphoridae" in various commonly used senses (excluding Oestridae s.lat.; including the Rhiniinae; excluding or including the Rhinophoridae; excluding or including *Mystacinobia*) does not appear to be monophyletic, though it does in trees having a much lower total fit, and in a very small fraction of the numerous trees 5 or 9 steps longer than the minimum-length trees. The assemblage (Rhiniinae + Toxotarsinae + Chrysomyinae) should likewise be rejected. A clade (Ameniini + *Euphumosia* + Phumosiinae + Mesembrinellinae + Parameniini + *Catapicephala*) is possibly Gondwanian. A clade (Toxotarsinae + Chrysomyinae + Calliphorinae + Luciliinae + Melanomyiinae) contains all the sarcosaprophagous "blowflies". The clades (Auchmeromyiinae + Bengaliinae) and (Chrysomyinae + Toxotarsinae) are the best supported groups. The group (Tachinidae + Sarcophagidae) is possibly the monophyletic sister-group of the Rhiniinae. *Mystacinobia* emerges from the analyses as the sister-group of all other Oestroidea and the Axiniidae as the sister-group of all the non-mystacinobiine oestroids. Although *Mystacinobia* is a guano-feeder in all stages, the biology of the other basal oestroid taxa indicates that reproductive association with small, living or dead, invertebrates is a primitive oestroid pattern.

01-107

PROPOSALS FOR THE PHYLOGENETIC CLASSIFICATION OF THE EMPIDOIDEA, OR ORTHOGENYA (DIPTERA, EREMO-NEURA)

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This is a discussion of how the current and partly conflicting hypotheses about the phylogeny of the Empidoidea and Eremoneura are to be translated into a consistent phylogenetic classification. The Empidoidea, including the Atelestidae or not, are likely to be a monophylum and the sister group of the Cyclorhapha. On the other hand, there is little doubt that the traditional concept of Empididae is paraphyletic. It is obligatory to subdivide the Empidoidea into monophyletic groups, the categorical ranks of which should be chosen consistently with those to be accepted within the Cyclorhapha. The same rank should be given to respective sister groups and to taxa of approximately equal geological age. In consequence, the number of families will have to be drastically reduced within the Cyclorhapha, whereas it may have to be increased in the Empidoidea. Thus, some or all of the conventional subfamilies in the Empididae and Hybotidae sensu Chvála 1983 may deserve the rank of families.

01-108

PHYLOGENY OF THE LOWER BRACHYCERA (DIPTERA):
MORPHOLOGICAL AND MOLECULAR EVIDENCEB. M. Wiegmann, D. Yeates¹, S.C. Tsaur²

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Phylogenetic relationships among the earliest lineages of the Brachycera have been difficult to resolve based on morphological features alone. Particularly contentious are relationships among the major subgroups of the superfamilies Stratiomyoidea, Empidoidea and Asiloidea. For example, at least six alternative hypotheses have been raised for relationships among families of the Asiloidea. Here we review and reexamine evidence on the higher-level phylogeny of the 'orthorrhaphous' Brachycera. Nucleotide sequence data may shed new light on relationships of the basal Brachycera. We have sequenced large portions of three genes, phosphoenolpyruvate carboxykinase (PEPCK), 18S ribosomal RNA, and 28S ribosomal RNA for taxa spanning the diversity of non-cyclorrhaphous Brachycera. In combined and separate phylogenetic analyses of morphological and molecular data we examine the strength of support for competing hypotheses of relationship for lower brachyceran lineages. Emphasis is placed on the position of the Bombyliidae, monophyly and relationships of the Asiloidea, and the position of several critical taxa including *Heterotropus*, *Hilarimorpha*, and Pantophthalmidae.

01-110

DIVERSITY OF ANTENNAL SENSORIA IN THE BASAL
CLADES OF THE THYSANOPTERA

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The possible origin of the diverse sensoria on antennal segments III-IV in the two families Merothripidae and Aeolothripidae (Thysanoptera, Insecta) is examined. An hypothesis of polarity of relationships between 18 genera - relating to both number and shape of the antennal sensoria - is provided.

01-109

THE IMPACT OF FOUNDING NUMBER AND
KLEPTOPARASITISM ON THE SEX RATIO OF A EUSOCIAL AND
A NON-EUSOCIAL GALL-FORMING AUSTRALIAN THRIPS SP.
(THYSANOPTERA: PHLAEOTHIRIPIDAE) ON *ACACIA*
*CAMBAGEI*B.D. Kranz, M.P. Schwarz¹, B.J. Crespi², L.A. Mound³

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The thrips *Kladothrips ellobus* and *K. hamiltoni* form galls on *Acacia cambagei* in the Lake Eyre basin of Australia. *K. ellobus* forms disc-like galls of about 1.5cm in diameter. Single generations of thrips are reared in these galls and mortality, due to invasion by the kleptoparasitic thrips, *Koptothrips xenus*, is high. *K. hamiltoni* forms tubular galls of about 3cm in length, where at least two generations of thrips are reared. The first generation comprises females and males which are micropterous, have enlarged fore-femurs and reduced antennae, and who act as soldiers in defence of the gall occupants. This species is threatened by invasion of *Koptothrips flavicornis* and an undescribed species of *Koptothrips*. Galls of *K. ellobus* and *K. hamiltoni* are founded by either a single female or a male and a female. The variation in sex ratios of eggs, nymphs and adults of these gall-formers, following both gall-induction and invasion, are quantified. Reasons for these variations, and differences between species, are discussed in the context of queen-worker conflict and defence strategies.

01-111

MISLEADING METHODOLOGIES IN THRIPS TAXONOMY:
HOSTS, HABITATS, HYPOTHESES AND MENSURATION

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Thrips host and habitat records that are based on single individuals may indicate little more than that thrips are highly vagile. To be useful to other biologists interested in host-plant and habitat ranges, both for economic and academic purposes, such records need to be based on the presence of larvae; that is, on those plants without which a thrips population cannot regenerate. Similarly, in descriptive taxonomy, data accumulation with little consideration of thrips as three-dimensional objects varying in time and space can produce misleading results. For example, head or setal lengths to 1% precision, with means to 0.05% precision, ignore the fact that specimen orientation on a microslide precludes such accuracy. Such data, accumulated without the conscious objective of facilitating comparisons, is part of the traditional approach to descriptive taxonomy that needs reconsideration. As in any other branch of science, taxonomy should involve the creation and testing of hypotheses, but to do this methodologies must be purposeful and standardised. The failure of so many taxonomic hypotheses concerning thrips, with more than 20% of species now being placed into synonymy, results from inadequate comparative methodology. The objective of this talk is to provoke a discussion of how thrips taxonomy might become more purposeful.

01-112

GENETIC VARIATION OF *THRIPS NIGROPILOSUS* WITH A DISCUSSION OF ITS TAXONOMIC TREATMENT (THYSANOPTERA: THIRIPIDAE)

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Thrips nigropilosus is widespread in the Northern Hemisphere, and females of this thrips exhibit a wing polymorphism. Recently, it was revealed that arrhenotokous and permanently thelytokous populations occur in Japanese *T. nigropilosus*, and that the former is divided into 3 groups with different genetic components of brachyptery. No genetic variability has been detected within the thelytokous thrips. Thus *T. nigropilosus* consists of 4 groups in Japan.

In this article, morphological characters, esterase zymograms, and partial nucleotide sequences of CO I and 16S rRNA genes of mitochondrial DNA were analyzed in adult females of the 4 groups, to clarify phylogenetic relationships among them and their taxonomic status. The arrhenotokous females were different from the thelytokous ones on development of sculpture on the ninth abdominal tergite and esterase zymograms, whereas the arrhenotokous females of the different groups could not be discriminated from each other by these characters. Sequence differences among the 4 groups were 0–3.6% in the CO I gene and 0–1.3% in the 16S rRNA gene. Molecular phylogenetic trees of the CO I nucleotide sequences revealed that females from the 4 groups are divided into 2 major clusters: arrhenotokous and thelytokous populations. These results suggested that the arrhenotokous populations with different genetic components of brachyptery are classified as a monophyletic group, and that the arrhenotokous populations and the thelytokous one should be recognized as separate species.

01-114

THE COMPLEX OF PHILIPPINE *Gynaikothrips* SPECIES

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Gynaikothrips are medium-sized to large thrips distributed mainly in the Orient with a few species known from the new world. There are six known species of *Gynaikothrips* in the Philippines: *Gynaikothrips uzeli* Zimmermann, *Gynaikothrips luzonensis* Priesner, *Gynaikothrips capitulatus* Reyes, *Gynaikothrips pedanus* Reyes, *Gynaikothrips pontis* Reyes and *Gynaikothrips xynos* Reyes.

The species of *Gynaikothrips* in the Philippines can be grouped into four: 1) *uzeli* group: *G. uzeli* 2) *luzonensis* group: *G. luzonensis* 3) *pedanus* group: *G. capitulatus* and *G. pedanus* 4) *pontis* group: *G. xynos* and *G. pontis*. The first three groups possess adult characters which definitely placed them under *Gynaikothrips* while the *pontis* group possesses characters that grade from those of adult *Gynaikothrips* and *Gigantothrips*.

Genera *Gynaikothrips* and *Gigantothrips* were described by Zimmermann in 1900. Members of these two genera are indistinguishable except for the longer intermediate antennal segments and body, additional pairs of wing retaining and accessory setae, and presence of foretarsal tooth in *Gigantothrips* species.

01-113

RELATIONSHIPS BETWEEN THYSANOPTERA AND THE REPRODUCTIVE AND VEGETATIVE PARTS OF *PROTEA* SPECIES (PROTEACEAE) FROM THE DRakensBERG MOUNTAINS OF SOUTH AFRICA

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On five species of *Protea* L. in the summer rainfall region in the south east of Africa, were recorded 37 species of Thysanoptera. The Terebrantia with 23 species in 16 genera were almost twice as abundant as the Tubulifera (8 genera, 14 species). Similarly, of the total of fifteen species in the inflorescence, only 3 belonged to the Tubulifera. The most abundant species on the leaves of these proteas was an unidentified *Scirtothrips* species, together with far less numerous members of another 3 species of Thripidae and one Phlaeothripidae. The *Scirtothrips* sp. fed regularly on the young leaves of *Protea caffra*. Only *Megalurothrips sjostedti* (Thripidae) could be found on both foliar and reproductive parts of these plants.

While species of Thripidae are quite likely to be involved in pollination by entering the florets already in the bud stage, members of the Tubulifera appear only much later and probably have no role in the pollination process. Thrips, together with other small florivore insects, can achieve high pollination rates of over 50%. However, the germination ability of these seeds in *P. caffra* is strongly reduced when compared with seeds from out-crossed pollination. Our results, compared with those from the Cape Floristic Region, reveal a very different thrips assemblage, with Tubulifera being dominant in the Cape but not in the Drakensberg mountains.

01-115

HOW TO CLASSIFY THE SPECIES OF THE GENUS *THRIPS* LINNAEUS (INSECTA: THYSANOPTERA).

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The genus *Thrips* Linnaeus 1758 is one of the largest genera in the order Thysanoptera comprising about 220 species world-wide. It is difficult to prepare for this genus a useful key to species based on easily recognizable features. It is much more difficult to work out a key to species based on characters which will allow phylogenetic groupings of the species.

Reduction of certain features or morphological dispositions are regarded as apomorphic. In any group of species such reductions occur in different combinations of characters. On the other side, the same species exhibit ancestral features also in different combinations.

In order to get an approximate phylogenetic line of the species of each group, the question has to be discussed which of the ancestral (plesiomorphic) characters are „older“ than other ones, and in contrast, which of the apomorphic characters are „younger“ than others. For an example, the hairy *Thrips minutissimus* Linnaeus 1758, a common European inhabitant of deciduous trees, shows plesiomorphic as well as apomorphic features. Does the species belong in its group to the ancestral representatives or to the advanced ones? Further details will be discussed.

01-116

CLASSIFICATION AND PHYLOGENY OF THE NEUROPTEROIDEA: AN INTRODUCTION

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Presently three orders are integrated within Neuropteroidea (= Neuropterida): Megaloptera, Raphidioptera, and Neuroptera s. str. The hypothesis of a sistergroup relationship (M+R)+N has been the predominantly accepted one. Arguments for an alternative hypothesis: (M+N)+R are presented. The new approach is mainly basing upon the larvae.

Aquatic larvae of the Megaloptera have so far been interpreted as an autapomorphy of this order, and aquatic larvae of the neuropteran Nevrothidae and Sisyridae as apomorphy of each family respectively. The new hypothesis interprets aquatic larvae of both orders as a plesiomorphic state, deriving, however, from a common ancestor, thus being a synapomorphy of M+N. Terrestrial, cryptonephric larvae within Neuroptera s. str. consequently are secondary.

In addition: Due to the morphology of larval mouthparts in Megaloptera evolving of neuropteran sucking tubes seems to be more parsimonious from a common ancestor of M+N than from a common stem species of the N+R.

As a consequence of the turned character polarity of aquatic/terrestrial larvae changed sistergroup relationships within Neuroptera s. str. result: (Nevrothiformia + Myrmeleontiformia) + Hemerobiiformia - within the Hemerobiiformia the Sisyridae being the adelphotaxon of all the other families.

01-118

THE METATHORACIC GLAND SYSTEM IN THE EVOLUTION OF THE FAMILY MYRMELEONTIDAE (NEUROPTERA)

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The Metathoracic Gland System of antlions consists of at least one pair of glandular reservoirs opening between the metathorax and the 1st abdominal segment, and in some cases a functional unit for secretion distribution. The latter is comprised of the pilula axillaris on the hindwing together with a pouch-like structure on the 1st abdominal segment. The secretion of the metathoracic gland purportedly acts as a male sexual pheromone. Interestingly, the gland system occurs in females as well, albeit in a reduced state.

For several species of Myrmeleontidae from different tribes, data are presented on the structure of the Metathoracic Gland System. It is argued that this set of organs forms part of the family's ground plan. Morphological peculiarities of its components may provide phylogenetically useful information to elucidate the still poorly resolved myrmeleontid phylogeny. Other exocrine gland systems confined to males are known in antlions, but in many taxa, only one source for pheromones appears functional. In some groups, however, males possess more than one type of well developed glandular organs, suggesting highly elaborated mating systems. Furthermore, secretions from reduced metathoracic glands of females might play an additional role in courtship. Glandular condition in females may nevertheless be interpreted as rudimentary, originating from a situation of equally developed Metathoracic Gland Systems in both sexes serving another function, such as defence.

Methods relevant for the investigation of exocrine glandular structures as presented here include scanning and transmission electron microscopy, and analytical techniques for the study of secretion chemistry. Preservation methods for material used in the investigation of different traits of the Metathoracic Gland System and of exocrine glands in Neuroptera in general are outlined.

01-117

THE CLASSIFICATION OF THE MYRMELEONTIDAE: CURRENT PROBLEMS AND ATTEMPTS TOWARDS A SOLUTION

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The current classification of the Myrmeleontidae recognizes three subfamilies: Palparinae, Stilbopertyginae (two Australian genera with nine spec.) seem to be beyond dispute at present, the arrangement of the other two subfamilies is controversial. The Palparinae include with certainty only the tribe Palparini, whereas the position of the Palparidiini seems to be questionable. Indisputable tribes belonging to the Myrmeleontinae are the Myrmeleontini, Acanthaclisini, Dendroleontini (all worldwide) and the Brachynemurini, Lemolemini and Gnopholeontini in the New World. The current construction of the Myrmecaelurini, Nesoleontini, and Nemoleontini is controversial and characters to delimit the Glenurini from the Nemoleontini and Gepini from the Myrmecaelurini are discussed. The reinstatement of Glenurini and Gepini is proposed.

01-119

AN OVERVIEW OF THE CURRENT STATUS OF CHRYSOPIDAE (NEUROPTERA) SYSTEMATICS

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Despite considerable progress in chrysopid systematics in recent years there are still many problems to be resolved. The relationships between the tribes and subfamilies of Chrysopidae are largely undetermined at present and monophyly of some, such as the Nothochrysinæ, have not been demonstrated. These problems are examined and a phylogeny for the chrysopid tribes and subfamilies is proposed. The relationships between the genera within the tribes and subfamilies are still unclear in many cases. This is particularly problematic in the Chrysopini, which includes nearly half the chrysopid genera. The systematics of the Nothochrysinæ and Chrysopini is reappraised and the value of certain morphological characters in resolving these problems are discussed. New work on courtship songs has revealed the presence of morphologically indistinguishable "song-species" in *Chrysoperla*, and the limitations of reliance solely on morphological characters to distinguish species. The implications of this in traditional taxonomic studies of Chrysopidae are briefly discussed.

01-120

THE *CHRYSOPERLA CARNEA* COMPLEX

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There is growing evidence that the holarctic common green lacewing, "*Chrysoperla carnea* Stephens", in fact consists of a number of valid sibling species and "song taxa". One of the best tools to separate and identify biological species in the genus *Chrysoperla* Steinmann is the analysis of the species specific vibrational courtship signals, which are performed by males and females in a duet as a precursor to copulation. At least ten more or less cryptic "song taxa" of the *carnea*-complex have been analyzed in the USA (3 spp.), in Europe (4 spp.) and Asia (5 spp.), and the number is growing yearly.

The splitting of a widespread species of such ecological and economic importance raises problems for science (how to interpret previous research findings?), biological control (mass rearing and release: the right species?), as well as conservation and law (shipping and introducing foreign species). An international "Working Group *carnea* complex" (WGcc) was established to investigate the taxonomy, systematics, biology and ecology of the various sibling species. A summary of present knowledge is given in the talk.

01-122

NEUROPTERA OF AUSTRALIA: ORIGINS, DIVERSITY AND RELATIONSHIPS

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The Australian Neuroptera fauna is large (>600 species) and diverse (14 families), with many archaic lineages and high levels of endemism. Recent comparative studies on the Neuroptera of Papua New Guinea, Indonesia and Malaysia have helped to clarify the peculiarities of the Australian fauna. Ancient Gondwana elements can be defined clearly, and the groups originating from the Indo-Malayan region are also largely definable. Biogeographical relationships of Australian Neuroptera are categorised and discussed in the perspective of current distribution patterns and centres of diversity.

01-121

SPECIES COMPLEXES AND SIBLING SPECIES IN CHRYSOPID LACEWINGS (NEUROPTERA, CHRYSOPIDAE)

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A number of sibling species has been discovered in recent years in chrysopid lacewings, mainly using three distinct approaches: 1) genetic structure by isozyme markers (Bullini & Cianchi, 1984; Cianchi & Bullini, 1992); 2) courtship call patterns (Henry, 1983, 1985); 3) microhabitat and phenology (Thierry *et al.*, 1992, 1995).

In the present note, the *Chrysoperla carnea* and *Mallada prasinus* complexes are reviewed. Genetic differentiation and allopatric versus sympatric modes of speciation in the two species complexes are discussed.

01-123

THE EXTANT DISTRIBUTION OF THE ORDER RAPHIIDOPTERA: A BIOGEOGRAPHICAL AND PHYLOGENETIC PERSPECTIVE

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The extant distribution of the Neuropteroid order Raphidioptera is restricted to certain arboreal parts of the Holarctic. It covers probably all arboreal regions of the Palearctic, in the Nearctic, however, it is confined to the south and southwest of North America, although northern and eastern parts of the continent would offer ecologically perfect conditions. The southern hemisphere presently lacks Raphidioptera totally.

There have been numerous efforts to explain this peculiar distribution pattern, but only recent results coming from various disciplines and from a reconsideration of relationships within the two families of the order (particularly Raphidiidae) have yielded sufficient arguments for the following assumptions: (1) The distribution of Raphidioptera during the Mesozoic must have been much wider and has included tropical and subtropical parts of the southern hemisphere, (2) the extant snake-flies of America are the remnants of ancestors having reached (South) America before the separation of South America from Africa and (3) by far most of all Raphidioptera have died out at the end of the Cretaceous except those which already had adapted to a temperate or a cold climate.

01-124

PHYLOGENY OF THE MOST PRIMITIVE LEPIDOPTERA:
THE STATE OF THE ART

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Relationships between the four basal clades within the Lepidoptera are arguably still best represented as Micropterigidae + (Agathiphagidae + (Heterobathmiidae + Glossata)), though both the Agathiphagidae and the Heterobathmiidae are known to possess apparently plesiomorphic traits unknown elsewhere in the 'order'.

The basal clades within the Glossata have now been found to constitute a highly resolved 'Hennigian comb':

Eriocraniidae + (Acanthopteroctetidae + (Lophocoronidae + (Neopseustidae + (Exoporia + Heteroneura)))). This phylogeny necessitates that reduction of the adult moth's mandibular musculature (and hence development of an adectic pupa) has occurred twice independently: in Lophocoronidae and in Neolepidoptera (= Exoporia + Heteroneura). On the other hand it permits us to continue to consider the development of an intrinsic proboscis musculature to be a unique event in lepidopteran evolution, hence a groundplan autapomorphy of the Myoglossata as first defined (= Neopseustidae + Neolepidoptera).

While the monophyly of the Heteroneura now appears reasonably well supported, the basal splitting events within the clade remain debatable. The arrangement Nepticuloidea + (Incurvarioidea + (Palaephatoidea + Tischerioidea + Ditrysia)) may tentatively be suggested on the basis of pheromone chemistry/female calling behaviour and eggshell ultrastructure.

As presently understood the basal lepidopteran cladogram indicates that the 'soil-animal' lifestyle of exoporian larvae is secondary. It cannot without ad hoc hypotheses be reconciled with the interpretation of an Upper Jurassic moth as belonging to the Glossata.

01-126

ADVANCES IN THE PHYLOGENETIC STUDY OF THE
ZYGAENOIDEAH. Geertsema, C.M. Naumann¹, G.M. Tarmann², M.E. Epstein³,
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Nine families, Zygaenidae, Limacodidae, Megalopygidae, Somabrachyidae, Dalceridae, Chrysopolomidae, Epipyropidae, Heterogynidae and Cyclotornidae are usually included in the Zygaenoidea. The higher classification of the Zygaenoidea presently is unsatisfactory due to the lack of characterisation(s) by clearly defined automorphies of the constituent taxa. Some families are well defined by one or several automorphies but also share a great number of plesiomorphic characters and their relationship(s), inter as well as in some cases intra, remains problematic. More recently, some revisionary studies, and which are reported here, have resulted in a better understanding of the constituent taxa, establishing an improved basis for a possible phylogenetic classification of the Zygaenoidea.

01-125

PHYLOGENY AND BIOLOGY OF THE LOWER
DITRYSIA (TINEOIDEA, GRACILLARIOIDEA
AND YPONOMEUTOIDEA)

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Although the three superfamilies discussed do not form a monophyletic group, they are customarily positioned together on the basis of several plesiomorphies at the base of the ditryisian Lepidoptera. Over the past decade investigations by several researchers have helped to clarify many relationships within each superfamily. Results of these studies are reviewed together with the biology and phylogeny of the currently recognized families. These include: (Tineoidea) Tineidae, Eriocottidae, Acrolophidae, Arrhenophanidae, Psychidae; (Gracillarioidea) Roeslerstammiidae, Douglassiidae, Gracillariidae; and (Yponomeutoidea) Yponomeutidae, Ypsolophidae, Plutellidae, Glyphipterigidae, Heliodinidae, Bedelliidae, Lyonetiidae. Also discussed are several problematic taxa for which family relationships still remain uncertain.

01-127

PHYLOGENY OF DITRYSIA

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The phylogenetic schemes previously proposed by the author (from 1988 to 1994) are reassessed and compared with a few other recent assumptions, which were based on either morphological or molecular data. While the latter tend to support the author's views, certain morphologically based works lead to fairly different proposals, such as a polyphyletic nature of the Obtectomera, a diphyletic nature of the Macrolepidoptera or a sister group relationship between the Gelechioidea and Apoditrysia. However most of these innovations prove quite unacceptable since they are based on inaccurate evidence. The morphology of the imaginal labial palps apparently supports the Yponomeutoidea/Gelechioidea sister group relationship, which had already been suggested from studies of the pupae. A number of new clades are tentatively proposed within the non-macrolepidopteran Ditrysia after examination/re-examination of several insufficiently known characters (related to - e.g. - larval morphology, imaginal internal anatomy and wing base region). The morpho-anatomy of several moths representing "relict taxa" is shown to be of fundamental importance, and the necessity of weighting characters is advocated to obtain reliable phylogenetic reconstructions.

01-128

DEFINITION AND RELATIONSHIP: KEY WORDS FOR STUDIES IN THE PYRALOIDEA (LEPIDOPTERA) DURING THE LATE 20TH CENTURY

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The Pyraloidea are one of the larger superfamilies of the Lepidoptera and many are pests to a wide array of crops and stored products. The two families, Pyralidae and Crambidae, comprise about 16,000 species worldwide. The delineation and relationships of higher-level taxa within the Pyraloidea have progressed rapidly during the latter part of this century, although activity at the species level has also exploded with the publication of major species lists from the Western Hemisphere and Australia.

The concept of the Pyraloidea has been refined tremendously during this century, although its sister-group relationship to other superfamilies remains unsolved. The definition and sister-group relationship of the Pyralidae and Crambidae based on adult and immature characters are reviewed. Studies on the subfamily relationships within each family are briefly reviewed. Recently, the use of characters associated with the tympanal organs on the first abdominal segment have been found to be useful in the delineation of taxa at various taxonomic levels in the Crambinae and Pyraustinae.

01-130

FOSSIL BUTTERFLIES IN PHYLOGENETIC PERSPECTIVE

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Fossilized butterfly remains are exceptionally rare. To date, 38 nominal species have been distinguished. The oldest known butterfly fossil is about 55 My old. In the lower Oligocene (about 35 Mya) all extant families are present. The known fossils stem from 11 sites in Europe, two in the U.S.A., one in Japan and one in Africa. The latter two sites are of Pleistocene age, the other sites range from Pliocene to lower Eocene. All body parts may be preserved, but the wings (with their venation) are predominant. The supposed taxonomic position is usually based on a comparison with an extant, modern species and conclusions are drawn without regard to the plesiomorphous or apomorphous nature of the similarity. Based on a critical evaluation the following number of fossil species can be assigned to family: Hesperidae - 1, Papilionidae - 5, Pieridae - 5, Lycaenidae - 2, Nymphalidae - 17, while the position of eight species remains uncertain. These numbers do not mirror the differences in species diversity among the modern butterfly families. The fossils are not helpful in solving problems with the higher classification of the extant butterflies. They do not contribute to the question of the origin of the butterflies either, except for the observation that the origin must have been before the Eocene.

01-129

PHYLOGENETIC PROBLEMS AND PROSPECTS IN THE MACROLEPIDOPTERA

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Results of recent phylogenetic studies of family interrelationships within the four large superfamilies of Macrolepidoptera are reviewed. The position of Pieridae within Papilionoidea, as sister-group of either Papilionidae or Nymphalidae + Lycaenidae, remains contentious. Geometroidea is expanded to include Sematuridae and Uraniidae. Within Noctuoidea, the monophyly of Noctuidae is questioned; the catocaline group of subfamilies may be more closely related to Arctiidae + Lymantriidae than to the noctuine subfamily cluster. Within a restricted concept of Bombycoidea, two clades are recognized, centred on Saturniidae and Sphingidae.

Future analyses need to be more objective and integrate data from immature stages and DNA/RNA with that from adult morphology if they are to further resolve and clarify macrolepidoptera family and superfamily relationships.

01-131

NUCLEAR GENES FOR LEPIDOPTERAN SYSTEMATICS

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DNA sequences from five nuclear genes have been tested for their ability to resolve relationships among lepidopteran clades. These sequences encode dopa decarboxylase, elongation factor-1 α , *Per* locus, phosphoenolpyruvate carboxykinase and 18S ribosomal DNA. To date, phylogenetic analysis has focused on sub- and infra-ordinal relationships within non-ditrysian Lepidoptera, and on relationships within Noctuoidea (particularly Heliothinae) and Bombycoidea (particularly Saturniidae: Attacini and Sphingidae). Identifying an assortment of nuclear genes whose evolutionary rates differ should prove useful for resolving numerous relationships within the Lepidoptera, and beyond.

01-132

PHYLOGENETIC RELATIONSHIPS IN *YPONOMEUTA* (LEPIDOPTERA, YPONOMEUTIDAE) AND THE EVOLUTION OF INSECT-PLANT ASSOCIATIONS

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Progress in phylogenetic methodology and molecular biology, allowing for the reconstruction of phylogenetic relationships from molecular data, has facilitated the growing recognition of the significance of a historical perspective in ecology. The evolution of present day insect-host plant associations can be best understood by establishing phylogenetic relationships (from molecular, allozyme or morphological data sets) among extant species and subsequently mapping their host plants onto the insect cladogram.

Several provisional phylogenies of a variable number of *Yponomeuta* species are available, based on frequency data at some 50 allozyme and general protein loci and on DNA sequences of nuclear and mitochondrial genes.

Celastraceae (species of the genus *Euonymus* in particular) served as host plants of the common ancestor of *Yponomeuta* and still serve as host plants for the majority of *Yponomeuta* species. In the evolutionary past at least once a shift has been made to the taxonomically and phytochemically unrelated Rosaceae and further onwards to Salicaceae; another shift occurred to Crassulaceae. *Yponomeuta* is not an exception to the rule that in phytophages sequential evolution, i.e. the shift of insect herbivores onto pre-existing plant species, is more common than coevolution.

The genus seems to be committed to extreme specialization per se rather than to a particular group of plants: whatever shift they have made they remain monophagous; the oligophagous *Y. padellus* is the only exception. Specialists may be committed to remain so, not because of any selective advantage, but simply because they have little or no evolutionary opportunity to reverse the process due to constraints on the evolution of the insects' nervous system or physiology (e.g. to detoxify secondary plant compounds). Current phylogenetic evidence, however, indicates that there is no intrinsic direction to the evolution of specialization.

01-134

THE GENUS *CHIRODICA* GERMAR: A MEDLEY OF LEAF BEETLES (COLEOPTERA, CHRYSOMELIDAE)

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The flea beetle genus *Chirodica* was established by Germar in 1834 for the species *chalconera* from South Africa (Cape Province). Afterwards, other 8 species by three different authors were attributed to this genus: Baly (1876: *elongata*, *fulva*, *fulvipes*, *wollastoni*), Jacoby (1900: *fulvicornis*, *puncticollis*) and Bryant (1924: *bicolor*; 1959: *capensis*). Subsequent contributions, as Scherer (1983), did not modify substantially the taxonomic situation of this genus.

Through the examination of the typical series of all species and of a large amount of material preserved in African and European museums, or directly collected by me, I had the opportunity to see that some *Chirodica* species are instead belonging to subfamily Galerucinae or to different Alticinae genera.

So, the systematic position of all taxa hitherto attributed to the *Chirodica* genus is reviewed and discussed. Finally, a new diagnosis and new taxonomic arrangement of this flea beetle genus are supplied.

01-133

PATTERNS OF DISTRIBUTION IN DUTCH LEAF BEETLES OF THE SUBFAMILY GALERUCINAE (COLEOPTERA: CHRYSOMELIDAE)

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One of the Dutch projects in the European Invertebrate Survey is the mapping of the distribution of Chrysomelidae. From the species of the subfamily Galerucinae preliminary maps are available.

The maps are based on the study of museum and private collections and on data made available by amateurs. Some conclusions already can be made.

Analysis of these maps give information on present and historical distribution patterns. The distribution patterns will be discussed. Causes for different patterns and fluctuations are partly natural and partly anthropogenic. An attempt will be made to correlate them to different life history strategies of the species.

Studies like these can give useful information for nature conservation strategies, both for the planning of locations as for the planning of management.

01-135

PROPOSAL FOR A NEW SUPRA-SPECIFIC CLASSIFICATION OF THE *CHRYSOMELA* GENUS AND THE OTHER RELATED GENERA (COLEOPTERA, CHRYSOMELIDAE)M. Biondi & M. Daccordi¹

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A proposal for a new supra-specific classification of the *Chrysomela*, *Plagiodera*, *Linnaeidea*, *Gastrolina* and *Gastrolinoides* genera based on a select set of systematically significant morphological characters is presented. Every character considered is critically analysed and discussed within the subfamily Chrysomelinae.

On the basis of a bi-state character matrix and using multivariate statistical analyses, a "dendrogram of affinities" was obtained. Its interpretation has allowed to consider in new way the taxonomic arrangement of this genera complex.

Finally, to support the proposed hypothesis of classification an analysis of its biogeographical implications is supplied.

01-136

THE PUPAE OF CHRYSOMELOIDEA AND THEIR USE IN PHYLOGENY (COLEOPTERA)

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Pupae or published descriptions of representative species of the Cerambycidae, except Anoploderminae, Philinae and Oxypeltinae; the Bruchidae, except Eubaptinae, Kytorhininae and Rhaebinae; and all chrysomelids, except the Orsodacninae, Megascelinae and Aulacoscelinae were examined.

Only those characters not influenced by either larval or adult morphology were chosen as phylogenetic indicators.

The pupae of Cerambycidae and Chrysomelidae are similar in many respects. Cerambycid pupae differ since they sometimes have gin-traps, apparently lack antennal papillae, always have functional spiracles on abdominal segment 5, but lack them on abdominal segment 8. The pupae of Lepturinae most closely resemble those of the Chrysomelidae and share the following characters in common: frons, vertex, clypeus, labrum and mandibles setose; apices of femora with setae; tarsi setose; abdominal segment 9 either with a single urogomph or paired urogomphi; abdominal tergites usually distinctly sparsely setose; usually functional spiracles on abdominal segments 1-5 (rarely missing on abdominal segment 5); pupation in earthen cell. Bruchid pupae resemble those of leaf beetles, especially the Criocerinae. Both are often whitish; head, body and legs glabrous; antennae usually with papillae; often with short, paired, sexually dimorphic urogomphi; indistinct spiracles on abdominal segments 1-6, but an additional reduced pair on abdominal segment 7 in criocerines; pupation usually in a whitish cocoon, either thin papery or thicker, this apparently produced from a mouth exudate; cocoon usually on host plant or in soil. The Sagrinae-Donaciinae; Zeugophorinae-Megalopodinae-Palophaginae; Synetinae-Eumolpinae; Clytrinae-Cryptocephalinae-Chlamisinae-Lamprosomatinae; Chrysomelinae-Galerucinae-Alticinae; and Hispinae-Cassidinae are grouped together on pupal characters.

A discussion of the significance of pupal setae, urogomphi, position of pupation and 'silken' cocoon construction in the Chrysomeloidea is given.

01-138

THE TAXONOMIC SIGNIFICANCE OF RECENTLY DISCOVERED IMMATURE STAGES, HOST PLANTS AND LIFE HISTORIES OF ISCADIDA CHEVROLAT (COLEOPTERA: CHRYSOMELIDAE: CHRYSOMELINAE)

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Since its inception in 1843, the southern African chrysomeline genus *IsCADida* Chevrolat has been associated with the genus *Timarcha* Latreille, regarded as its counterpart in the Holarctic Region. Currently these two genera are classified in the Chrysomelini: Entomoscelina and Timarchini: Timarchina, respectively, but this is not yet regarded as conclusive.

The adults, immature stages, host plants and life histories are well known for *Timarcha*, the only genus in the tribe Timarchini. The subtribe Entomoscelina contains 27 known genera, distributed throughout the world. Of the eight genera that occur in the Afrotropical region, seven are endemic to southern Africa. Nothing was known of the host plants of these endemic genera until recently, when intensive collecting resulted in the discovery of immature stages and host plants for three *IsCADida* species. Life cycles for these were unravelled in the laboratory.

IsCADida is now known to be associated with plants of the genera *Impatiens* (Balsaminaceae) and *Rhoicissus* (Vitaceae). Neither of these plants nor the Balsaminaceae as a whole have previously been recorded as hosts for the Chrysomelinae. Discovering the eggs of *IsCADida* allows an assessment of the extra-chorion ultrastructure of the Entomoscelina, the only subtribe in the Chrysomelinae for which it is unknown. Preliminary studies of the immature stages of *IsCADida* revealed the following interesting features: eggs are deposited singly on the ground or apparently on the host plant; larvae have mandibles with six teeth, distinct tubercles, eight abdominal spiracles, tarsal claws with an inner tooth, long setae and egg bursters associated with two long setae on the meso- and metathorax and first abdominal segment; three larval instars occur and the life cycle is either bi- or multivoltine. Most of these characters support the current placement of this genus in the Chrysomelini: Entomoscelina.

Results obtained in this study indicate that the characters of the immature stages and host plants of the ancestral genus *Timarcha* clearly differ from those of *IsCADida*. This supports the position of the latter genus in the more highly evolved tribe Chrysomelini.

01-137

STUDIES ON ASIAN ALTICINAE GENERA BASED ON WING VENATION, GENITALIA, AND METAFEMORAL SPRING (COLEOPTERA: CHRYSOMELIDAE)

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As part of a continuing study on the establishment of true generic groups in this largest chrysomelid subfamily, four independent internal character systems were studied (i.e. hind wing venation, male and female genitalia, and the metafemoral spring). The authors have studied these character systems in over 60 Asian genera, primarily from eastern Asia and adjacent areas. Many genera are associated preliminarily based on morphological similarity of the character systems. Some of these associations support the traditional catalogue arrangement (i.e. presumed close relatives) (e.g. *Sangariola* and *Laboissierea*, or *Hyphasis* and *Hemipyxis*) while others do not (e.g. *Eudolia*, *Garuda* and *Licyllus*, or *Phygasia*, *Liprus*, *Lipromorpha*, and *Pseudoliprus*). Such studies are combined with previous research to show other similarities and differences among genera from other regions. These studies will be used to establish phylogenetic relationships among genera in the future when a significant proportion of the subfamily has been studied.

01-139

THE ROLE OF CHEMICAL SIGNALS FOR PREDATORS AND PARASITES OF EGGS AND LARVAE OF GALERUCINAE (COLEOPTERA: CHRYSOMELIDAE)

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Eggs and larvae of leaf beetles of the subfamily Galerucinae, tribe Galerucini, are known to contain anthraquinones and anthrones, which were proved to act as feeding deterrents against ants and birds. However, the presence of these compounds does not prevent the eggs from parasitism. Eggs of *Galeruca tanacetii* and *Galerucella* species are often heavily attacked by the chalcidoid wasps *Oomyzus galerucivorus* and *O. gallerucae*, respectively. Our studies on the role of chemicals in host selection of these parasitoids will be presented. When investigating host finding, the attractivity of volatiles from the following sources were studied in olfactometer bioassays: gravid leaf beetle females, plants with host eggs, undamaged plants, plants damaged by feeding leaf beetles, and faeces of the beetles. Host recognition behavior of the parasitoids is indicated by frequent antennal drumming on the surface of host eggs. Our studies aimed on the isolation, characterization and identification of (extra)chorion compounds inducing antennal drumming. Host acceptance as indicated by oviposition of the parasitoid might be mediated by compounds within the host eggs. GC-MS analyses revealed that anthraquinones and anthrones are not located at the outside of the eggs, but are only present inside. The parasitoid does not transfer these compounds from the larval to pupal stage: anthraquinones and anthrones were only found in the excreta of the last larval instar, but not in pupae and adult parasitoids. The role of chemicals for predators and parasites of the galerucine juvenile stages investigated will be balanced against the possible importance of physical cues.

01-140

HOST-PLANTS OF THE ENTOMOSCELINA REITTER
(COLEOPTERA CHRYSOMELIDAE CHRYSOMELINAE)

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The Entomoscelina, according to Daccordi (1994) integrating the Phyllocharina, actually comprise 29 genera divided into 3 sections. It is very difficult to find clear cut common characters using only the external morphology of the adults. In addition, very little is known regarding the feeding habits and the larvae. Some genera are orophilous and nocturnal, some feed on vines and bushes, other live near the ground or on herbs at the Southern tips of Australian, African and American continents. Probably, the subtribe Entomoscelina is artificial and composed of many divergent taxa. The food-plants of 14 taxa are examined here and as expected they do not have any plant taxa in common: Vitaceae, Lamiaceae, Brassicaceae, Urticaceae, Scrophulariaceae, Bignoniaceae, Pittosporaceae, Verbenaceae. However, the related food-plants within the Scrophulariaceae and the Verbenaceae for *Chalcomera* s. lat. confirms the unity of the genus through South-East Asia, Australia and New Zealand. Three genera feed on Verbenaceae: *Phyllocharis*, *Didosoma* and *Phola*.

01-142

BIOLOGY AND BEHAVIOR OF BRAZILIAN LEAF BEETLES
ASSOCIATED TO SOLANACEOUS PLANTSLenice Medeiros¹, David N. Ferro² & Agenor Mafra-Neto³

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We monitored the population dynamics and behavior of several chrysomelids associated with native solanaceous plants of Ijuí, RS, Brazil. *Platyphora quadrisignata*, *P. fasciatomaculata*, *P. vinula*, *Gratiana* sp. 1, *G.* sp. 2, *Metriorhiza elatior*, *Charidotella* sp., *Cistudinella* sp., *Lema reticulosa*, *L. apicalis*, and *L.* sp were studied. All were monophagous, except *P. vinula*, which was associated with both *Solanum megalochiton* and *S. sanctae-catharinae*. The chrysomelids diapaused during the winter (July and August). From October to December, larval recruitment peaked; this coincided with low parasite and predator population densities. Eulophidae and Asopinae caused high mortality in Criocerinae eggs and larvae, respectively; Tachinidae and Asopinae caused high mortality in Chrysomelinae and Cassidinae larvae. Predation and parasitism of immatures increased throughout the season, peaking during January. Certain reproductive strategies of Criocerinae and Chrysomelinae may have evolved to ensure protection against the ubiquitous presence of egg parasites and predators. The Criocerinae *Lema apicalis*/*Lema reticulata* (one species?) laid egg masses in a clumped distribution creating hard-to-find, but dense local populations. In contrast, the Chrysomelinae were all larviparous, bypassing the less protected sessile egg stage. Chrysomelinae larvae are protected by cycloalexic behavior or by shifts in foraging period from days to nights. Criocerinae larvae are protected by a coat of regurgitate and feces coupled with cycloalexic behavior. Cassidinae larvae are protected by a highly mobile shield made of exuvia and feces. Although well protected, the Chrysomelids in Ijuí still suffered large losses of immatures to parasites and predators.

01-141

PHYLOGENETIC STUDIES ON THE LARVAE
OF THE CHRYSOMELIDAE (COLEOPTERA)

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The larvae treated in this study include 77 species in 45 genera and 16 subfamilies. The phylogenetic relationships among subfamilies were inferred on 34 characters of the larvae by the Wagner tree method on a computer after the evaluation of every character and the inference of its polarity.

The cladogram obtained by this method suggests the phylogenetic position of the Orsodacninae and Megalopodinae on a first branch as sister taxa from the rest. Next branch is the Bruchidae, which should be treated as a subfamily of the Chrysomelidae. The Donaciinae constitute the third branch. The remaining subfamilies can be divided into two phylogenetic groups, one comprises the Zeugophorinae, Criocerinae, Synetinae, Eumolpinae, Chlamisinae, Clytrinae, Cryptocephalinae, and Lamprosomatinae, while the other includes the Alticinae, Galerucinae, Chrysomelinae, Cassidinae and Hispinae.

01-143

IS HOST-PLANT DERIVED CHEMICAL DEFENSE IN
CHRYSOMELA AN EVOLUTIONARY CONSTRAINT?J.M. Pasteels, Ph. Soetens, D. Daloze⁽¹⁾

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Most *Chrysomela* species feed on Salicaceae and their larvae secrete in their defensive glands salicylaldehyde derived from plant phenolglucosides (mainly salicortin). This host dependence for defense does not constitute an insurmountable evolutionary constraint for further shifts on non-salicaceous plants.

Species of the subgenus *Microdera* feed on *Betula* and *Alnus*. Molecular phylogenies (in coll. with T. Hsiao) indicate that feeding on non-salicaceous plants is a derived condition within the genus.

Comparative analysis of the secretion of selected beetle species fed on *Salix* spp. devoid of phenolglucosides in their leaves (in coll. with S. Neuvonen) demonstrated that the larvae still produce a defensive secretion, which was reduced in volume and contained no or little amounts of salicylaldehyde. This contrasts with *Phratora vitellinae*, also secreting host-derived salicylaldehyde, which does not produce anymore a defensive secretion when fed on plants devoid of phenolglucosides. It remains to discover if the flexibility in defense of *Chrysomela* spp. is due to their ability to utilize a larger spectrum of plant precursors or to a capacity of autogenous synthesis. It is suggested that the selective pressure of specialist natural enemies could induce shifts on plants without phenolglucosides, even though defense against generalist predators is reduced.

01-144

THE CYTOTAXONOMY AND EVOLUTION OF CHRYSOMELINAE
LEAF BEETLES REVISITED.

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Twenty-two species of Chrysomelinae mostly from Europe, have been recently examined to increase the sample of cytogenetically known taxa of the subfamily to some 200 species. Eight species of *Chrysolina*, three of each *Oreina*, *Cyrtonus* and *Phratora*, two of each *Iscadida* (from S. Africa) and *Chrysomela*, and one of *Ambrostoma* (from NW China), have been analyzed. Among these data, *Oreina viridis* (Duft.) has shown the ancestral meioformula of 9+Xyp (2n=20), the most frequent in the plesiomorphous genus *Timarcha*, which stands off with regard to the other species of *Oreina*, all having 11+Xyp (2n=24). The possible chromosomal evolution of *Chrysolina* subgenus *Stichoptera* is discussed. The location of *C. fuliginosa* (Ol.) and *C. lepida* (Ol.) in the subgenus *Allochrysolina* is also proved since they share the meioformula of 20+Xyp (2n=42). Moreover, we point out the intermediate karyological derivation of *Iscadida* and most *Cyrtonus* with 12+Xyp (2n=26) and 13+Xyp (2n=28) respectively, and the advanced position of *Chrysomela* and *Phratora*, all whose species have 16+Xyp (2n=34). The subfamily has three main steps of chromosomal evolution set up by the the haploid numbers of: 10 in most taxa of *Timarcha*, in *Cosmogramma decora* and *Oreina viridis*, 12 in most *Chrysolina*, *Calligrapha*, *Gonioctena* and paropsines, and 17 in *Phaedon*, *Chrysomela*, *Phratora*, etc., which agrees with some other features of phylogenetic value.

01-146

COMPARATIVE INTERNAL HEAD CAPSULE MORPHOLOGY IN
CHRYSOMELIDAE (COLEOPTERA)

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Cuticular surface structures of the inner face of head capsules have not found much attention in insect morphology and phylogenetics up to now. Head capsules from about 20 species out of 8 leaf beetle subfamilies (Crucidae, Donaciinae, Sagrinae, Cassidinae, Clytrinae, Chrysomelinae, Galerucinae, Alticinae) and Bruchidae have been studied after maceration by means of a Stereo-Electron-Microscope. The diverse inner surface patterns yield promising characters for phylogenetic analysis.

01-145

TOPOGRAPHIC AND GENETIC STRUCTURE IN AN ALPINE LEAF BEETLE: *Oreina cacaliae* (COLEOPTERA, CHRYSOMELINAE).
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Herbivorous insects with their strong association to host plants often show very patchy distributions. On what scale "gaps" between suitable host plants or host plant patches are barriers to gene flow and affect population structuring depends on the dispersal of the species. Leaf beetles (Coleoptera: Chrysomelidae) feed as larvae and adults on the same host plants. Both, larvae and adults, often show an aggregative distribution and a low vagility. In willow feeding leaf beetles genetic differentiation was found already between beetles feeding on neighbouring trees. We were interested whether such a small scale differentiation can also be found in herbivores feeding on a less permanent resource. Therefore we studied the spatial population structure of *Oreina cacaliae*, an alpine species feeding on herbaceous host plants (*Adenostyles*, *Senecio*, *Petasites*). To gain information about population sizes and movement rates we observed marked beetles at three different sites over one season. Allozyme electrophoresis of 25 presumptive loci reveals for *O. cacaliae* high polymorphism, high inbreeding rates and considerable genetic variation at a very small scale. Significant FST values are found between groups only some hundred meters apart. This is in agreement with results from mark and recapture studies, which give high recapture rates and small movement rates (max. 3m/day). Such a local differentiation could not be explained by host plant use or by different altitudes. Geographic distances, however, are at larger distances (>10 km) well correlated with genetic distance. Obviously limited movement capabilities lead to either an extremely local differentiation (possibly in combination with strong habitat specificity) or to non random mating. It could be that we sampled family groups or closely related individuals who do not migrate.

01-147

THOUGHTS ON GALERUCINE ZOOGEOGRAPHY (COLEOPTERA,
CHRYSOMELIDAE)

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Galerucinae is one of the largest subfamilies within Chrysomelidae, with a worldwide distribution, but only a few of its genera are represented in more than one zoogeographical region. The subfamily has been further subdivided into tribes, and they into subtribes and genus groups; among these categories we already find some that are restricted to one region, while others are widely distributed. The subfamilies of Chrysomelidae seem mainly to have differentiated during the Jurassic period, when the continents were united in the Pangean supercontinent. When Pangea began to break up during the Cretaceous period, the Galerucinae were apparently already widely distributed and differentiated. Among the tribes Galerucini and Hylaspini seem to have a Laurasian origin, Metacyclini a West Gondwanan, and Luperini gives the appearance of already having been widely distributed at an early date. On the Indian subcontinent the Galerucinae are quite numerous and differentiated in the northern parts, particularly in the Himalaya. Further south there is a much lower number of genera, and almost all these genera are widely distributed. A large part of the Indian genera are also found in China and South-East Asia. Probably the Indian fauna is derived from the Eurasian continent; any Galerucinae living on the Indian plate before its drift to the north can be assumed to have perished in the Deccan volcanism at the end of the Mesozoic era. Our knowledge of Galerucine phylogeny is still insufficient for a definite zoogeographical analysis. For one thing the position of the New Zealand genera (*Adoxia*, *Allosterna* and *Bryobates*) should be ascertained, but many other questions also remain. Some of the recognized genera appear to be rather heterogenous, and we need a synthesis of studies from various regions.

01-148

HOST-PLANT SELECTION IN TWO SALICACEAE SPECIALISTS, *PHRATORA VITELLINAE* AND *PH. TIBIALIS*

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Food plant preferences in nature and in the laboratory were studied for two species, *Phratora vitellinae* and *Ph. tibialis*. Ten different potential host plants were included in this study. Five willows: *Salix alba*, *S. babylonica*, *S. caprea*, *S. fragilis* and *S. purpurea* and five poplars: *Populus deltoides*, *P. deltoides* x *nigra*, *P. nigra*, *P. trichocarpa* and *P. trichocarpa* x *deltoides*. Phenolic glucosides, mono- and disaccharides content, as well as trichome density of the leaves were quantified.

Ph. vitellinae is oligophagous on plants with leaves rich in phenolic glucosides and with low trichome density. *Ph. tibialis* is monophagous in the field on *S. purpurea*, but in laboratory preference tests, *S. babylonica* is also accepted as a food-plant.

Ph. vitellinae larvae are able to use plant phenolic glucosides (salicortin and salicin) as precursors of defensive secretion but not tremulacin and tremuloidin. Salicortin in synergy with sucrose had a strong phagostimulatory effect on the adults of *Ph. vitellinae* but a more complex mixture of phenolic glucosides and sugars is even more stimulant.

Chemical defense of *Ph. tibialis* larvae is not host-derived but autogenous. In adults, only the mixture of salicortin and sucrose is found to be stimulant. This result does not explain the monophagy of the species.

In both species, flavonoïds present in willow leaves (7-O-eriodictyol glucoside, 7-O-luteoline glucoside and 7-O-naringenine glucoside) did not stimulate nor inhibit feeding in laboratory tests.

01-149

THE DISTRIBUTION OF THE SENSILLA ON THE HIND WINGS OF THE FAMILY CHRYSOMELIDAE (COLEOPTERA) AND ITS SYSTEMATIC SIGNIFICANCE

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The distribution of the sensilla on the hind wings of the family Chrysomelidae (Coleoptera) and its systematic significance. The sensilla chaetica (s.c.) on the so-called recurrent vein (Rv), which consists of M1+2, M3+4, and m-m, of the hind wings in 348 chrysomelid species of 181 genera belonging to 19 subfamilies, was studied (Table). The variability and the systematic significance of this new character are discussed.

Subfamily	No. gen.	No. Spec.	No. M s.c.
Orsodacninae	1	2	1~ 2
Zeugophorinae	1	3	0~ 2
Megalopodinae	4	4	2~10
Aulacoscelinae	2	6	1~ 7
Sagrinae	11	19	0~ 8
Donaciinae	4	22	1~ 6
Criocerinae	4	15	0~ 9
Synetinae	2	2	1~10
Chrysomelinae	13	25	0~14
Galerucinae	33	46	0~ 4
Alticinae	69	128	0~ 4
Clytrinae	2	6	1~ 6
Cryptocephalinae	4	16	0~ 3
Chlamisinae	2	3	0
Lamprosomatinae	3	4	1~ 4
Hispinae	4	6	1~ 7
Cassidinae	6	15	0~13
Megascelinae	1	4	1
Eumolpinae	15	23	0~ 7
Total	181	348	

01-150

DIVERSIFICATION OF FECAL SHIELD-BEARING LEAF-BEETLES (CHRYSOMELIDAE) PROMOTED BY HOST PLANT-DERIVED CHEMICAL DEFENSE

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Predators and parasitoids may represent a major selective force that promotes diversification in herbivorous insects. Mechanisms by which this might occur are not well understood but dietary specialization, enforced by predation pressure, appears to be a requisite condition. Larvae of criocerine leaf beetles (*Lema*, *Neolema*, *Oulema*), and of an alticine (*Blepharida*), due to the anatomical oddity of a dorsal anus, deposit feces that form a 'shield' on top of their bodies. Appropriately host-derived fecal shields are repellent to ants and function as a defense. Extraction and fractionation of plant and frass, followed by the ant bioassay of fractions, revealed that larvae expropriate the secondary chemicals of their hosts and incorporate them, apparently with little transformation, into their frass. Furthermore, larvae concentrate specific host compounds 1000 fold. A repellent plant terpenoid (mol. wt. 296) was isolated from all shields and a complex of steroidal glycoalkaloids was isolated from shields of the solanaceous feeders.

The simple terpenoid defense is augmented in larvae feeding on more chemically complex hosts. The criocerine clade colonizing the more complex solanaceous hosts is derived and is twice as diverse as the sister taxon that retained the ancestral hosts in the Commelinaceae.

Chemical complexity of the shield defense with respect to targeting and efficacy against a suite of enemies (i.e. does a more complex shield afford protection against a broader spectrum of enemies?) and its relationship to host specialization and enhanced beetle diversification will be examined to test the predator-free space hypothesis.

01-151

GENETIC VARIABILITY IN MONO- AND OLIGOPHAGOUS CHRYSOMELID BEETLES AT VARIANCE WITH THE NICHE-WIDTH VARIATION HYPOTHESIS.

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The niche-width variation hypothesis (Van Valen, 1965) predicts a positive correlation between niche breadth and genetic variability. A group of animals ideally suited for testing the hypothesis is found in the phytophagous insects, in which the niche is mainly determined by the number of hostplant species they live on. In this study we compare genetic variability between mono- and oligophagous *Phyllotreta* species (Coleoptera : Chrysomelidae).

In total 36 populations belonging to 10 different *Phyllotreta* species were analysed for 10 loci using allozyme electrophoresis. Genetic variability was studied using four different criteria : average number of alleles per locus, % of loci polymorphic, observed heterozygosity and expected heterozygosity under Hardy-Weinberg conditions. For each of the measures a Mann-Whitney U test was used to check for significant differences between mono- and oligophagous species. For all the genetic variability measures used, monophagous species show a significantly higher genetic variability compared to the polyphagous species. This is at variance with the niche-width variation hypothesis. Two alternative explanations are possible, the first concerning the limited availability of hostplants in monophagous *Phyllotreta* species, the second concerning the relation between oligophagous species and agricultural crops.

Van Valen, L. 1965 *Am.Nat.* 99 : 377-390.

01-152

HOST PLANT SELECTION OF PHYLLOTRETA SPECIES.
THE CASE OF PHYLLOTRETA VITTULA (REDT.)

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Nearly all species of Phyllotreta feed on crucifers or on the plants of related genera of Resedaceae and Capparaceae. The one known exception is Phyllotreta vittula (Redt.) which feeds on grasses and cereals but, however, accepts various crucifers too. Feeding responses of adults were evaluated by means of leaf disc test. Significant differences were found between responses to eight plants of Gramineae and five crucifer plants. Strong preference of grasses was detected. The origin of this preference provides material for evolutionary studies on the host plant selection not only of Phyllotreta species, but phytophagous insects as well. Further studies are needed to clarify the biochemical background of the host plant selection of Phyllotreta vittula (Redt.).

01-154

HOST PLANTS AND THE DIVERSIFICATION OF
NEOTROPICAL TORTOISE BEETLES (COLEOPTERA:
CHRYSEMELIDAE: HISPINAE)

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Phylogenetic relationships among tribes and genera of Neotropical tortoise beetles ("cassidoid Hispinae", sensu Borowiec) are examined using a cladistic analysis of 85 morphological and behavioral characters. Modern host plant associations, mite associations, habitat preferences and antipredator defenses are used to infer historical aspects of diversification in these leaf-feeding beetles.

01-153

DISTRIBUTION PATTERNS OF CHRYSEMELID BEETLES IN
CENTRAL AFRICAN FORESTS

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In several types of forests in Central Africa, the arthropod fauna of selected tree species was investigated using insecticidal fogging technique. In a gallery forest in East Rwanda, on eight trees of Teclea nobilis (Rutaceae), Chrysomelids reached 42% of all beetle specimens. Especially a few species of Alticinae were highly abundant, and most of them found on all trees. In a mountain rain forest in West Rwanda, on nine trees of Carapa grandiflora (Meliaceae) Chrysomelids, predominantly Galerucinae, were much less abundant and the frequency of the species was lower. In a semi-deciduous forest in North-West Uganda 64 trees assigned to four species (Cynometra alexandri (Caesalpiniaceae), Rinorea ardisiifolia (Violaceae), Trichilia rubescens (Meliaceae), and Teclea nobilis) were fogged. Eumolpinae formed the bulk of Chrysomelids. The diversity of leaf-beetles was relatively equal on the different tree species in primary as well as in secondary forest, but differs stronger between these two forest types.

01-155

REDUCTIONS AND NEW INVENTIONS DOMINATE
OOGENESIS OF STREPSIPTERA

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The endoparasitic life of strepsipterans, especially of larviform females, reduces to great extent external and internal organs. Light and electron microscopic investigation of ovaries confirm this general feature:

1) Somatic tissues of ovaries like outer envelope, the ovary ligament, the tunica externa of ovarioles, the terminal filament, somatic apical cells, prefollicular cells, the pedicel and the lateral oviduct are reduced totally. 2) A previtellogenic growth phase of oocytes is absent. Growth of euplasm is shifted into the phase of cluster mitoses, before morphological differentiation of oocytes becomes apparent. 3) Nurse cells remain diploid and their cell membranes degenerate during onset of vitellogenesis. 4) Vitellogenesis is reduced, vitellin and fat vacuoles contribute to only 50% of the final volume of the egg. 5) Chorionogenesis is reduced and produces a very thin chorion.

However, some features of normal development remain and allow to consider the type of ovary as strongly deviated, but nevertheless, polytrophic meroistic: 1) Germ cells undergo synchronized, incomplete divisions which follow the 2ⁿ rule: one sibling cell gets all old intercellular bridges, the other none. 2) During the division program, all cystocytes form a rosette with a polyfusome. 3) Only one cell of each cluster will be determined as oocyte, all others serve as nurse cells. 4) Each cluster is surrounded by some somatic cells, serving as follicle cells.

New inventions are 1) Fission of clusters during the phase of cluster mitoses; thus, the amount of oocytes raises tremendously. 2) Protection of the germinal vesicle, while nurse cell nuclei will degenerate in the same cytoplasm.

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01-156

SPERM ULTRASTRUCTURE IN STREPSIPTERA.

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In the last few years, the fine structure of the spermatozoon in five species of Strepsiptera, namely *Xenos moutoni*, *X. vesparium* (Stylopidae), *Elenchus tenuicornis* and *E. japonicus* (Elenchidae) and *Halictophagus chilensis* (Halictophagidae) has been examined.

The spermatozoon of all species studied so far is a filiform cell in which the common two parts, sperm head and sperm tail, are recognizable. The sperm head consists of a monolayered acrosome resting on an elongated nucleus which contains an eccentric portion of uncondensed chromatin, except in the sperm of *X. vesparum*. The sperm tail shows a 9+9+2 axoneme flanked by two mitochondrial derivatives. The connecting piece between the head and the tail is characterized by a bilocular head-tail junction.

In spite of this general organization, the spermatozoon ultrastructure of Strepsiptera has taxonomic value showing features which can be considered as generic or specie-specific. The spermatozoon of the two species of Stylopidae has some peculiar characters such as, a brush-like glycocalyx, U-shaped mitochondrial derivatives, while, sock-shaped mitochondrial derivatives are typical of the two species of Elenchidae examined. In addition, the bilobate acrosome of *X. moutoni*, the very long nucleus without the eccentric portion of uncondensed chromatin as well as the U-shaped mitochondrial derivatives without paracrystalline material of *X. vesparum*, may be considered as specie-specific characters.

01-158

LITTLE BUGS THAT LIVE IN SMALLER BUGS AND SO AD INFINITUM
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Intercellular procaryotes in mycetocytes, follicular cells and mature eggs have been described in a wide variety of insects and here the presence of *Wolbachia*-like organisms in five species of Strepsiptera is presented. This is the first report of the presence of micro-organisms in Strepsiptera. *Wolbachia* has been recorded in the hosts of Strepsiptera, namely Hymenoptera, Diptera, Orthoptera, Homoptera and Hemiptera.

These intracellular associations have major consequences for both bacteria and host, and in this study three organisms are involved: bacteria, host (strepsipteran), and superhost (host of strepsipteran). Cytoplasmically inherited micro-organisms are responsible for a number of reproductive disorders in insects and other arthropods. In Strepsiptera the micro-organisms are present in both sexes during the larval stages, but only in the neotenic female and not the male adult or pupa. The micro-organisms are mainly present in the germ cells/oocytes/eggs in the female, which indicates that the micro-organisms might be maternally transmitted. In the larval stages of the male the micro-organisms are present in the gut region.

In one system of Strepsiptera studied so far - the Delphacidae and their strepsipteran parasite *Elenchus*, the evidence indicates that there might be horizontal transmission.

01-157

SYSTEMATICS OF NORTH AMERICAN STREPSIPTERA, WITH NOTES ON THE BIOLOGY OF *CAENOCHOLAX FENYESI*

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Systematics of North American Strepsiptera reviewed for the first time since Bohart's revision in 1941. Bohart synonymized ten *Xenos* species into two currently recognized species. Evidence is presented that one or more of these species are valid and should not have been synonymized. The genus *Stichotrema* is reported from the United States for the first time. A female and triungulin of *Caenocholax fenyesi* Pierce are described. This is the first record of a female in this genus. Females have been described for only ten percent of known species of Myrmecolacidae.

The biology of *C. fenyesi* and the relationship to its known host, *Solenopsis invicta*, are discussed. The distribution of *C. fenyesi* suggests that *S. invicta* is not its only host and this relationship is possibly the result of a recent host switch in the United States. Stylopization levels are very low in colonies of *S. invicta* in the United States. This may be a result of the proposed recent host switch or the scarcity of its female host. A behavior change resulting from stylopization is documented. A behavior change occurs in *S. invicta* in late stages of strepsipteran development, causing abnormal behavior, including a loss of normal social behavior.

01-159

FACTORS AFFECTING THE REPRODUCTIVE POTENTIAL IN STREPSIPTERA

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In phoretic insects, the successful life cycle is maintained depending on the probability of the first instar larvae which can attain to the proper hosts. They are well known to produce enormous number of eggs throughout their long life span. Strepsiptera has the highest reproductive potential in Insecta except some social insects such as termites, etc. It is not exaggerated to say that female stylopids are ovipositional machine.

The egg complement (total number of eggs produced in a maternal body) greatly differs among the species of Strepsiptera, maximum was found in *Strichotrema dallatorreanum* (750,000 eggs) and minimum in *Triozocera macrosotyti* (984 eggs). The difference of egg complements in Strepsiptera might be arisen from both the external-factors (life type of hosts, etc.) and the internal-factors (species and size of hosts, sex of hosts, number of stylopids parasitized on a single host, etc.). The author analyzed factors to determine the egg complement in various Japanese species of the following genera, *Triozocera* and *Blissoxenos* (Corioxenidae); *Halictophagus* (Halictophagidae); *Elenchus* (Elenchidae); *Stichotrema* (Myrmecolacidae); *Pseudoxenos*, *Xenos*, *Paraxenos*, *Halictoxenos* and *Stylops* (Stylopidae). The results were generally concluded as follows, irrespective of their phylogenetic relation: 1) Egg complement was larger in those species of which hosts were gregariously inhabiting in a very limited area or conspicuously visiting flowers than that in those species of which hosts are weakly having above two traits. 2) Egg complement was reduced largely by the parasitization on small-sized hosts and male hosts, as well as by polyparasitism as compared with that obtained from the parasitization on large-sized hosts and female hosts, as well as by monoparasitism.

01-160

THE FIRST INSTAR LARVAE OF THE STREPSIPTERA

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Current investigations with scanning electron microscopy techniques (SEM) include first instar larvae of all until now known families of the Strepsiptera, except Callipharixenidae and Bohartillidae. Semi thin sections of two taxa were examined with light microscopy.

The prognathus head is flat on its ventral, convex on its dorsal side. In ventral view head appendages are visible. They are the maxillae and the labium, each bearing paired palps. In several taxa the labial and maxillary palps are almost reduced. Antennae are absent. The fusion of lateral and ventral margins of the maxillae and labium with ventral margins of the head capsule produces a preoral cavity. This cavity contains the mandibles and, at its caudal side the primary or inner mouth opening. The secondary or outer one is at the apex of the front end of the head. A tentorium is present. In lateral view 2–6 stemmata are visible on each side. The so called “Antennenfelder” (“antenna areas”), are paired sensory pits, which are not present in all taxa. In the apomorphic ones they are reduced to very small pits. A basic pattern of setae is varied in the several families.

The legs consist of coxa, trochanterofemur, tibia, and tarsus. The tarsi of the first two pairs of legs are mostly modified into suction pads, the hind ones are rod shaped. The intercoxal sternites are very variable.

The abdomen is 10-segmented, with two long movable caudal setae. These are homologous to cerci. The plesiomorphic ones, characterized by fused terminal segments, can jump. The apomorphic ones, with free terminal segments lost this capability.

Evolutionary trends that are visible in the first instars are discussed.

01-162

NOVEL MORPHOLOGICAL CHARACTERS AND THEIR IMPACT ON CURCULIONOID CLASSIFICATION

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One of the areas of classification of the Curculionoidea which is exciting most interest is in the ‘higher’ weevils, particularly among those subfamilies of Curculionidae which might be sister-group to the Scolytinae. Setting aside disputes over ranking of the subfamilies/tribes concerned, there are few data which enable cladistic resolution of this part of the Curculionidae. The stridulatory mechanisms employed by weevils, and the presence and structure of specialised organs along the anapleural suture, ‘sclerolepidia’ both shed light on the classificatory problems in question, as well as being potentially valuable in other areas of weevil classification.

01-161

FROM MOLECULES TO EMBRYOS: INSIGHTS ON THE PHYLOGENY AND EVOLUTION OF THE STREPSIPTERA.

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Phylogenetic relationships among the holometabolous insect orders were inferred from cladistic analysis of nucleotide sequences of 18S ribosomal DNA (83 exemplars), 28S rDNA (52 exemplars) and morphological data (176 characters). Twenty insect outgroups, all holometabolous insect orders, and six strepsipteran species were included in this study

Cladistic analysis supports a sister-group relationship between Strepsiptera and Diptera (=Halteria). This relationship is well-supported, as indicated by large Bremer support values, large branch lengths, and a lack of sensitivity to the parameters used in phylogenetic reconstruction. Long-branch attraction does not appear to influence this result because the strepsipteran-dipteran branch lengths and sequence divergences are comparable to other well-supported monophyletic groups included in the analysis.

Homeotic transformation of strepsipteran thoraces is suggested as a hypothesis to account for the presence of halteres on different thoracic segments in Diptera and Strepsiptera. The progress of studies underway to determine the pattern of homeotic gene expression in strepsipteran embryos is presented.

01-163

SOLVING THE RIDDLE: COMBINING LIFE-HISTORY ANALYSIS AND MORPHOLOGICAL COMPARISON IN WEEVIL PHYLOGENETICS

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Albeit that the phytophagous feeding habit of weevils (Coleoptera: Curculionoidea) is in many instances manipulated for bio-control practices against invasive alien plants and the pollination of beneficial plants, it is the magnitude of their destruction of crops and other cultivated plants that label them as one of the most economically important insect groups. Despite the fact that the higher classification of the weevils has now been sorted out, the group is still rife with many unresolved phylogenetic “grey-areas” on the lower taxonomic levels. Many regional and a few world revisions of certain weevil groups have been cladistically researched, but all in all the current knowledge of weevil faunistic relationships on the generic-specific level across continents is still in disarray. It is now necessary, as the next phase in the weevil classification saga, that this be addressed on an inter-continental basis. This paper discusses the value of considering bio-ecological traits, i.e. life-history studies and host-plant associations in conjunction with basic alpha taxonomic morphological comparison to determine certain weevil relationships and ultimately reach phylogenetic solutions. Female genitalic morphology of the Brachycerinae (Curculionidae), host associations of the Urodontinae (Anthribidae) and life-history analysis of the Somatodini, Entimini, Microcerini, Brachycerini and Rhytirhinini (Curculionidae: Brachycerinae) serve to demonstrate the point.

01-164

THE GENUS *PODAPION* RILEY, 1883 IN THE OLD WORLD: A NEW SPECIES AND BIOGEOGRAPHICAL IMPLICATIONS (COLEOPTERA: APIONIDAE: APIONINAE)

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The discovery of a new species of the genus *Podapion* Riley, 1883 in the mountains of Central Spain poses a very interesting biogeographical problem, already aroused with the discovery that the species described originally as *Magdalis mariae* Formánek, 1911, belongs to this genus too (Korotyaev, 1993). The generic description is amended, the new species *P. baenai* is described and figured and a key to the three known species is given.

Two different hypothesis on the phylogenetic history and the zoogeography of the species involved are presented. The first scenario shows a hypothetical common ancestor of the genus living in both Western and Eastern North America and Europe (age: Jurassic to Middle Cretaceous: not later than 95 MY), whose populations became split by the opening of the North Atlantic Ocean and began to diverge both genetically and morphologically. The American population split up again into two populations (western and eastern) because of the severe cooling of climate during the Oligocene and Miocene, and the formation of the dry pasture belt across Middle North America, while the European population suffered harsh climatic conditions during Pleistocene glaciations and retired into Balcanic and Iberian refugia, speciating into the relictic *P. mariae* and *P. baenai*.

The second hypothesis differs only in that the supposed ancestor was present only in Euramerica (Late Cretaceous) and that the Eastern American population invaded Western America after the drying of the Mid-Continental Seaway in the Early Tertiary.

01-166

THE WORLD WEEVIL DATABASE PROJECT
(COLEOPTERA: CURCULIONOIDEA)

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Information concerning biology, ecology and systematics of larger insect groups is tremendous and widely scattered in the literature. Comprehensive up to date monographs on a worldwide scale are therefore rare or non-existing for many taxa. This makes it difficult even for the specialist to obtain relevant information needed for identification, biological, ecological or biocontrol work.

For this reason an efficient bilingual database system has been developed to handle pictures and text. The software can be used for any taxon. Additions made by different researchers can be combined for regular updates. Various types of lists can be printed or exported.

So far more than 27000 species and genera and 7000 synonyms of Curculionoidea have been included covering the fauna of the Americas, Australia and parts of Europe and Asia. Contained are distribution, host plants (where available), pest status, biological control potential and systematic status. These data are connected to a literature database with 5500 publications listed. Currently 700 full colour pictures illustrate typical representatives of common genera, biotopes and details for identification.

The phylogenetic arrangement of taxa on a worldwide scale has been tried in spite of sometimes contradictory evidence from literature. As much work will still be needed here, the database system has been devised to allow quick modifications.

Future perspectives include the completion of the data for the group studied on a worldwide scale. Another important goal is the assembly of determination keys to be used with the database.

01-165

EXTERNAL MALE SEXUAL CHARACTERS IN THE APIONIDAE (COLEOPTERA, CURCULIONOIDEA) AND THEIR PROBLEMATIC USE FOR PHYLOGENETIC ANALYSES

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Apart from the differences in rostrum shape, almost universal among Apionidae and being an evident female adaptation to the kind of plant, place and way of oviposition, male structures facilitating a firm grip on the female during copulation are main characters responsible for sexual dimorphism in this family. At least 19 major types of modifications can be recognised, which apparently approximates the number of "technically" possible solutions. The most common are modified tibial apices or (never simultaneously) undersides of basal tarsal segments, but such characters can be found also on coxae, femora, metasternum, abdominal ventrites, and even on antennae or aedeagus (examples illustrated). These characters are generally much more popular and diverse in apionids than in other weevil families, which may result from their very convex body and closely situated coxae.

Although diagnostically invaluable, such characters should be, however, cautiously used for cladistic analysis. Being not typical epigamic characters, with few exceptions (mucrones in *Trichapion* and *Apiotherium* complexes, specialised femoral areas in *Fallapion*) they do not undergo sexual selection (in Darwinian sense). For several reasons such structures are commonly homoplasious:

- a) a high selection pressure favouring appearance of any character of this kind, as directly influencing successful gene transfer of its possessor;
- b) morphological simplicity; they are easy to appear;
- c) functional limitations cause numerous repetitions of the same idea;
- d) uniform body shape in apionids leads to similar solutions;
- e) further complication or evolutionary change is unnecessary (unless female body shape changes considerably).

01-167

PHYLOGENETIC STUDIES ON INDIAN ZYGOPINAE WITH PARTICULAR REFERENCE TO THE STRUCTURE OF EXTERNAL GENITALIA (CURCULIONIDAE: COLEOPTERA)

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The members of subfamily Zygopinae mainly include stem borers and attack many forest and crop plantations. The various characters of taxonomic importance have been taken into account for clear understanding of disputed-related species.

Besides thorough sampling, identification and description of new and old taxa, a particular stress has been laid on the structure of genitalia as means of divergence and phylogenetic relationship within the taxa of the subfamily and its status vis-a-vis other subfamilies. The structure of genitalia in the studied species shows that it conforms to basic pattern characteristic of the family Curculionidae in general. It has been observed that the condition of endophallic armature in male, and shape and size of spermatheca in female show variations of phylogenetic significance. The details of the structure of phylogenetic importance will be presented.

01-168

PHYLOGENY OF GENUS *Sitona* Germar, 1817 (COLEOPTERA, CURCULIONIDAE).

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A phylogenetic study of the genus *Sitona* was made to test the present classification of this group, which includes three subgenera, one of them, the nominotypical subgenus comprises also several species-group (Sectio) as established by Reitter in 1903. New characters were studied, from male and female genitalia, including internal sac, mouth parts, proventriculus and metendosternite. A matrix was made including new and previously used characters to construct a cladogram. Results show that:
Subgenus *Charagmus* Sconheir, 1826 is well defined by several apomorphies.
Subgenus *Coelositona* González, 1971, previously considered monospecific, must include more species.
Subgenus *Sitona* Germar, 1817 is polyphyletic, some of the species are to be included in *Coelositona*, others must form a new subgenus and only the rest must remain in this subgenus. Only part of the groups defined by Reitter are supported by synapomorphies.
The new classification of *Sitona* shows consistence with recent data on phylogeny of Leguminosae based on analysis of chloroplast genome.

01-170

BARK BEETLE COEXISTENCE IN LEAFSTALKS: ECOLOGICAL SUCCESS IN A NUTRIENT-POOR HABITAT.

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A guild of bark and ambrosia beetles, longhorn beetles and zygopine weevils inhabits dead leafstalks of neotropical *Cecropia* species. In Costa Rica, of scolytids we found 11 specialist (*Scolytodes*) species and 14 generalists (6 genera); we also recorded 9 zypogine species and 2 cerambycids breeding in *Cecropia* petioles.

Our research has focused primarily upon the most common group, the scolytids. Fecundities of leafstalk scolytids are among the lowest for any animal; nevertheless, host specific species are very successful, and a mixture of host-specific and generalist species coexist in *Cecropia* patches. The taxonomy and biogeography of these beetles are presented, along with ecological and behavioral data. The leafstalk guild is proposed as a model system for studying ecology and evolution in patchy tropical habitats.

01-169

HOST AND REFUGE PLANTS OF COLEOPTERA CURCULIONOIDEA

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Knowledge of host plants of Curculionoidea is of paramount importance. Many taxonomists have apparently not given the right emphasis to it in the past. Refuge plants are here intended as those used by a species as feeding resource, mainly of adults, when usual hosts are not available. Efforts to find out host and refuge plants of Curculionoidea are notably important for taxonomy, evolution, applied entomology, and ecology. Most probably oligophagous weevils predators of epigeal parts of herbaceous plants, particularly annuals, are more subject to selection pressure. Multivoltine species should be forced to use additional hosts to survive when unfavourable conditions arise. Shifting from the usual host to another can be supposed relatively easier in them than in root or wood feeders, which have also longer life as a rule. Can shiftings of adults from a plant to another bring to an evolutionary event? Although this is presently not supported by experimental data on short-term period, it is possibly of considerable weight in a long-term one. It seems very important that scientists note relationships of any kind between weevils and plants, both in the larval and adult stage. This would lead to evidenciate sibling species, to discover different ways of life of adults and larvae, and to differentiate monophagous or oligophagous species from related presumable polyphagous ones. Since many weevils play a remarkable role as pests of cultivated plants or as biocontrol agents, investigation about their host and refuge plants may be a linking point between taxonomists and applied entomologists.

01-171

WHAT MAKES "COMMON" SCOLYTIDS COMMON?

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"Commonness" is discussed, for neotropical bark and ambrosia beetles. Successful tropical scolytids tend to be (a) both widespread and abundant, locally and regionally; (b) inbred, through regular brother-sister mating; (c) fungus-farming or breeding in twigs and seeds; and (d) host generalists. Common species are also sometimes "pests" in agriculture or forestry. Patterns in commonness are compared, for Costa Rican scolytids in primary, secondary, and planted forests, for certain "life styles", and for continuous vs. patchy habitats, and implications for conservation and forestry discussed. The presumed high levels of homozygosity in widespread and abundant tropical scolytids seems paradoxical in light of ("Red Queen") theories emphasizing the importance of genetic variability for competing in biotically complex environments.

01-172

PHYLOGENY AND EVOLUTION OF PARTHENOGENESIS IN THE GENUS *ARAMIGUS* HORN (COLEOPTERA: CURCULIONIDAE): A STUDY INTEGRATING MOLECULAR AND MORPHOLOGICAL DATA

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Aramigus Horn, which includes a number of parthenogenetic lineages, has been the subject of 2 recent phylogenetic studies, one using morphological characters and one using mitochondrial DNA (mtDNA). In the present study we integrate these two approaches.

We analyzed 39 terminal units (TU's) of *Aramigus* from which mtDNA and/or morphological data were available, and selected 32 morphological characters (21 informative) and 792 base pairs of mtDNA. Analysis of the morphological data left many relationships unresolved, especially within the *A. tessellatus* species complex. Analysis of the molecular data provided a better resolution. The strict consensus of all mtDNA and morphological MP trees was virtually unresolved.

We also conducted parsimony analyses for the 34 TU's from which both mtDNA and morphological data were available (M + M TU's) and we obtained 2 MP trees. One of these trees was completely congruent with one of the 14 MP mtDNA trees. Both MP trees showed a single topology for the *A. tessellatus* complex and implied 3-4 origins of parthenogenesis within the complex. When all 39 TU's were considered, there were 5 MP trees, differing only in the placement of the *biseriatus* morphotype of *A. tessellatus*. These 5 trees were all congruent with one of the 2MP trees for 34 TU's and implied 3-5 origins of parthenogenesis within the *A. tessellatus* complex. We interpret the single tree that was found by both the mtDNA and combined analyses as the best-corroborated estimate of maternal-lineage genealogy for *Aramigus*.

01-173

TRICHOGRAMMA TAXONOMY WORKSHOP: TOWARD A STABLE NOMENCLATURE FOR THE SPECIES OF TRICHOGRAMMA (HYMENOPTERA: TRICHOGRAMMATIDAE)

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Species of the genus *Trichogramma* are among the most widely used biological control agents in the world. Unfortunately, optimum utilization of these insect egg parasites has often been compromised by disagreement over the identity of some of the most common and important species. For example, there has been no unanimity among taxonomists as to the definition of such frequently studied species as *Trichogramma evanescens* Westwood, *T. euproctidis* (Girault), *T. cacoeciae* Marchal, and *T. brasiliense* (Ashmead). Most of the controversy is due to the absence or inadequacy of type material. The purpose of this workshop is to clearly identify the most important sources of disagreement and to recommend solutions that will insure nomenclatural stability. Workshop participants will include several specialists currently active in the taxonomy of the genus.

01-174

HOFFER-FLIES (DIPTERA: SYRPHIDAE) OF UZBEKISTAN
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One hundred feventy eight species of Syrphidae from 34 genera are known in Uzbekistan. The greatest amount of species occurs in mountain zone (115 species, or 89.8%). Fifty five species (42.9%) are known from the foot-hills zone. Twelve species (9.3%) are known in desert zone and 33 species (25.0%) - in the tugai zone of rivers. Fifty one species (39.8%) occurs environment which is under anthropogenic pressure. Forty seven species (39.0%) have aphidophagous larvae. Abundance, period of activity, number of generation, puculiarities of feeding, hibernation, and natural enemies of aphidophagous Syrphidae are discussed. Some new methods of artifical culturing of aphidophagous species are also discussed.

01-176

SYSTEMATIC REVIEW OF THE AFRICAN GENERA
PSAMMOTHERMA, *ANTENNOTILLA* AND *CTENOCERAEA*
(MUTILLIDAE, HYMENOPTERA), WITH REMARKS ON THE
SUBTRIBE SMICROMYRMINA

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Three genera of African mutillids, *Psammotherma* Latreille, 1805, *Antennotilla* Bischoff, 1920 and *Ctenoceraea* Nonveiller, 1993, are unique within the whole family for having a peculiarly modified antennal flagellomeres (all taxa known from male sex only).

In the course of the extensive revisionary studies on the Afro-tropical fauna, and particularly in connection with the discovery of *Ctenoceraea*, the examination of the type material resulted in revision of both older taxa, with significant redefinition of the concept of original *Antennotilla*. This primarily monotypical genus (*A. phoebe* Péringuey) should be broadened to include *Psammotherma cyanochroa* André, but also several other species (most of them apparently new) showing various degrees of antennal modifications, as different states of the same morphological trend (with *A. cyanochroa* having the most advanced and rather unusual one). The definite limit of this genus is still difficult to determine, since it involves the revision of a number of species, currently recognizable only as members of the large "genus" *Smicromyrme* sensu lato. The revised genus *Psammotherma* is morphologically rather homogeneous, comprising only 4 species, and *Ctenoceraea* is currently monotypical. Their antennal modifications are of two distinct forms, but both can be described as pectinate (the rare antennal type within Hymenoptera); no morphologically intermediate states have been recorded within the Mutillidae.

All the examined taxa belong to the *Smicromyrme*-group of genera (in Africa comprising also *Corytilla* Arnold, *Pseudocephalotilla* Bischoff, *Sulcotilla* Bischoff and only tentatively *Guineomutilla* Suarez, as well as some other female-based undescribed genera), which probably constitutes the most primitive section of the large and diverse subtribe Smicromyrmina (sensu Brothers, 1975). However, each of them represents an independent phylogenetic lineage within the group: *Ctenoceraea* being the most closely related to *Corytilla*, and two others to different subgroups of heterogeneous African "genus" *Smicromyrme* s.l. The true *Smicromyrme* Thomson is probably absent from the tropical Africa.

01-175

PHYLOGENETIC RELATIONSHIPS AMONG THE GENERA OF THE
SUBTRIBE GYMNOLEURINA (COLEOPTERA SCARABAEIDAE
SCARABAEINAE): PRELIMINARY RESULTS.

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The last important study of the subtribe Gymnoleurina was carried out by André Janssens (1940). The Belgian entomologist, considering external morphological characters only, enlisted 91 species and grouped them into four main genera: *Paragymnoleurus* Shipp, *Garreia* Janssens, *Allogymnoleurus* Janssens, and *Gymnoleurus* Illiger. A few new species have been described in recent years.

According to Janssens, Gymnoleurina have to be considered a homogeneous taxon. Species are distributed in Africa and Asia, reaching the islands of the Malaysian archipelago, Sumatra, Java, Borneo, and Philippines. Only a few species are known from southern Europe.

At this stage of our research - within the framework of the phylogenetic revision of the whole subfamily Scarabaeinae - we took into account 22 species belonging to the four generally accepted genera.

We considered 33 characters obtained from external morphology, epipharynx, male and female genitalia. On the basis of these characters we carried out a phylogenetic analysis, using different computer packages (PAUP, MacClade, and PHYLIP).

Characters were considered unordered, and trees unrooted, since a sufficiently reliable outgroup seems not to be available without a precise phylogenetic hypothesis concerning the whole subfamily.

Data hitherto processed produced several trees which show a quite good differentiation into natural groups, thus essentially confirming, with some poor rearrangements, the monophyly of the genera stated in current literature.

01-177

SYSTEMATICS, CLADISTICS AND BIOGEOGRAPHY OF *ENTOMODERES*
SOLIER, 1836 (COLEOPTERA: TENEBRIONIDAE: NYCTELIINI)

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Entomoderes comprises nine species that occur in South America, southern parallel 17 and are insects endemics to dry areas in central and northern Argentina and southern Bolivia, principally in Monte and Chaco formations. This genus is a monophyletic group, defined traditionally by the lateral expansions of pronotum. The genus is revised, providing redescrptions and illustrations of its species based on the external morphology and male and female genitalia, a key for identifying them, and distributional maps. Analysis of material from northern Argentina revealed a new species that is described. The cladistics analysis was based on 30 characters, and the polarity of them was determined using the genus *Pilobalia* Burmeister. Characters with more than one apomorphic state or not comparable with the outgroup were treated as nonadditive. Using the program Pee-Wee only one cladogram with best fit was obtained. The analysis shows that eleven new characters constitute synapomorphies of *Entomoderes*. The cladogram obtained shows two basal groups, first the sister species *E. erebi* and *E. cellulosus*, and a second monophyletic group constituted by the remain species, both groups supported by structures of pronotum and elytra. Only three characters show parallel or reversal evolution. Information on geographical distribution was used along the tree to analyze vicariant events. Three main areas of endemism could be determined for *Entomoderes* species: Chaco (2 species), southern Monte (2 species), and northern Monte (4 species). One species (*E. draco*) is widespread. The only vicariant event, that is shows by others taxa of Coleoptera (*Cnemalobus*, Carabidae; *Enoplopactus*, Curculionidae) is the separation of northern Monte with Chaco-southern Monte. These areas are separated by mountain chains in Salta, Tucumán, Catamarca and La Rioja provinces (Argentina).

01-178

EVOLUTIONAL PATTERN OF PSYLLID MALE REPRODUCTIVE SYSTEM(HOMOPTERA:PSYLLOIDES)

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The follicle number of male testes of psyllid was two in mode, which has 76% in the frequency, distributes worldwide, occur from the all families of this group. The numeric variation of follicle was one to twelve, and one follicle type comes from the Gondwanan region and the family Spondyliaspidae and Aphalaridae. Four follicle type is popular in the Palearctic and Oriental continent, and the genera Psylla, Eritriozia, and Cyamophila etc.

01-180

A SYSTEMATIC STUDY OF THE MESOCHORINAE(HYMENOPTERA : ICHNEUMONIDAE) FROM THE EASTERN PALEARCTIC REGION

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Taxonomic, morphometric, phenetic and cladistic analyses were performed using the subfamily Mesochorinae which collected from Eastern palearctic region and the following results were obtained.

As a result of taxonomic study, a total of 5 genera, 70 species was confirmed in the Eastern palearctic Mesochorinae. Among them, four species of *Astiphromma* and four species of *Mesochorus* are new ones. In addition, six species (*A. confusum*, *A. varipes*, *M. confusus*, *M. fuscicornis*, *M. rubranotatus*, *M. uniformis*) are new to Eastern palearctic regional fauna.

In order to clarify the interspecific variation of *Astiphromma jezoense*, a morphometric analysis was performed for seven populations using PCA (principle component analysis) and discriminant analysis. As a result of PCA, 25 quantitative characters are grouped into four factors. The characteristics on legs are especially important components both in male and in female. Morphometric analysis indicate that considerable morphological gap is correlated with geographical habitat.

In the phenetic analysis, clustering patterns which obtained from different methods using different numbers and characters represent the degree of interspecific morphological similarity. The results provide information to preparing useful key for the Eastern palearctic Mesochorinae.

A single parsimonious cladogram was obtained from cladistic analysis. The genus *Cidaphus* was the basal clade among five genera. Four other genera were branched in the order of *Astiphromma*, *Mesochorus*, *Stictopisthus* and *Plectochorus*. Among them, two genera *Stictopisthus* and *Plectochorus* form a sister group.

01-179

TAXONOMY AND PHYLOGENY OF THE SUBFAMILY TRYPHONINAE (HYMENOPTERA : ICHNEUMONIDAE)

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For the taxonomic and phylogenetic studies of the subfamily Tryphoninae (Hymenoptera : Ichneumonidae), specimens were collected from March of 1988 to September of 1995 in the southern part of Korean peninsula. Specimens were also obtained from various museums and laboratories both in Korea and in other countries. This research utilized about 1,000 domestic specimens and 3,010 foreign specimens including 10 specimens from North Korea.

Four tribes, 23 genera and 72 species a total is distributed in Korean peninsula including previously unrecorded eight genera and 24 species.

In order to get a desirable results from morphometric analysis, both PCA and discriminant analyses were performed. Morphometric analysis indicate that length of lateral carina of scutellum, width of face, width of clypeus, length of 2rm + 3rm and Length of Cul between Cua and 1mCu(fore wing) are important factors to classifying the subgenera. It could overcome the problems following subgeneric classification if these characters were incorporated into subgeneric classification system.

The cluster and discriminant analyses suggest that the Townes(1969) and Townes et al.(1992)'s systems are well fit for the classification of Tryphoninae. Cladistic analysis indicate that the Tryphoninae is a polyphyletic group and do not fit to the basic concept of natural classification because of its complex classification system. Tribes Sphintini and Ankylophonini, due to its host specificity, can be seen the results of convergent evolution.

According to zoogeographic studies, large numbers of species originating from the palearctic region and a large percentage of endemic species, I can assume that the Korean Tryphoninae crossed over from northern hemisphere.

The fossils were found at the Cretaceous amber located at the Siberia peninsula, Taimyr. I think it to be the dispersion center. The world-wide distribution of the Tryphoninae seems to coincide with the continental movement.

01-181

PHYLOGENY AND EVOLUTION OF THE STAPHYLINID TRIBE SCAPHISOMATINI (COLEOPTERA)

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Leschen and Löbl (1995) examined the phylogenetic relationships of the fungus feeding staphylinid subfamily Scaphidiinae (consisting of the tribes Cypariini, Scaphiini, Scaphidiini and Scaphisomatini). In their study, however, Scaphisomatini was treated superficially and was represented in the data matrix by two terminal taxa. We conducted a preliminary cladistic study of Scaphisomatini (which includes about 35 described genera) by including two genera from each of the subtribes (Heteroscapina, Baeoceridiina, Baeocerina, Scaphisomatina, and Toxidiina). This analysis, based on 11 terminal taxa (including a generalized outgroup) and 34 characters, resulted in three parsimonious reconstructions of relationships. Each of these show that all subtribes are polyphyletic with the exception of Baeoceridiina which in two trees are monophyletic and sister taxon of Scaphisoma (Scaphisomatina). These data suggest the current classification is unsatisfactory and must be changed to reflect natural groupings. Our results are interesting because most members of Baeoceridiina are associated with Old World termites suggesting that the evolution of social insect inquilineism may have evolved one time in the subfamily.

01-182

A TAXONOMIC STUDY OF THE JAPANESE SPECIES OF THE GENUS *FARISTENIA* (LEPIDOPTERA, GELECHIIDAE).

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The genus *Faristenia* was proposed by Ponomarenko in 1991 for the Russian species and she described seven new species. Park (1993) revised the genus with descriptions of three new species and redescription of five known species from Korea. Ponomarenko (1991) stated that the genus *Faristenia* is related to the genera *Hypatima* and *Dactylethrella* and Park (1993) placed the genus between them. In Japan five species belonging to the genus are recorded fragmentarily by Park (1993) and Ueda (1995), however, revisional study of the genus has never been done. After examination of Japanese *Faristenia*, I reached the following conclusions:

- 1). Ten species of the genus occur in Japan and some of them must be described as the new to science.
- 2). The monophyly of the genus is supported by the following synapomorphies: the presence of hair pencils at the basal portion of the cell on the hindwing in the male; the tegumen with inflated basal half in the male genitalia; the coiled ductus ejaculatorius in the male genitalia; the coiled ductus bursae in the female genitalia.
- 3). The short gnathos of the genera *Faristenia*, *Dendrophilia* and *Capidentalia* are considered to be apomorphic condition shared by these genera in the subfamily Chelariinae.

01-184

SYSTEMATIC AND DISTRIBUTIONAL STUDIES OF ASIAN *ANDRENA* (HYMENOPTERA, ANDRENIDAE)

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After Smith (1869) described *Andrena halictoides* from Japan, many species of the genus *Andrena* have been discovered and described. We have started to make a survey of this group in China mainland since 1993 and also have started to study materials collected from Japan, China, Korea, Formosa and Maritime Districts of Russia. We have described new species and have determined systematic positions of described species. In the present study we show outline of systematic and distributional studies of Asian *Andrena* with special references to principal subgenera, such as *Andrena* s. str., *Hoplendrena*, *Holendrena*, *Simandrena*, *Micrandrena*, etc.

01-183

THE EVOLUTION OF EGG CHARACTERS IN SOME TRUE BUGS (HEMIPTERA: HETEROPTERA: PENTATOMOIDEA)

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The evolution of egg characters in Pentatomoidea are traced from the fundamental characters that have true phylogenetic value. These characters have been separated from those that are temporary adaptations to specific environments.

The phylogeny of 23 genera of Pentatomoidea, based on embryogenesis, embryonic orientation, characteristics of the chorion, the number and size of the micropyle, location and thickness of the "T"-or-"Y"-shaped egg-burster, mechanisms of hatching, colour, and egg form shows, the genera *Elasmucha* and *Sehirus* as the probable outgroups.

Based on the above characters, the superfamily is divided into two groups: (1) Acanthosomatidae, Cydnidae, and Thyreocoridae; and (2) Pentatomidae and Scutelleridae. The first group, particularly Acanthosomatidae and Cydnidae, are ancient and primitive families.

The three forms of narrowed rounded ends, spherical, and cylindrical shell in the superfamily have evolved independently, and along different pathways from an ovoid ancestral egg.

01-185

AN ELECTROPHORETIC CHARACTERIZATION OF THE ITALIAN SPECIES OF *SIGARA* S. STR. (HETEROPTERA: CORIXIDAE)

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Some years ago, individuals belonging to the genus *Sigara* (subg. *Sigara* s. str.) (Heteroptera: Corixidae) were founded in Central Italy (Di Giovanni et al., 1984). These individuals seem to represent another species as regard to the similar species *S. dorsalis* (Leach) and *S. striata* (Linnaeus). The taxon, still named *Sigara* sp. is different from the other especially for the parameres morphology, size of the strigil and shape of the eight urotergite.

The objective of the present study is to investigate the biochemical differences and to describe the genetic structure of the different Italian populations of *S. striata* (La Muta Lake - Trentino A. Adige) *S. dorsalis*, (Belfiore - Veneto) *Sigara* sp. (Tresa stream - Umbria; Zittola swamp - Molise) using electrophoretic techniques. Alleles were distinguished by means of horizontal starch electrophoresis of homogenized specimens. Genetic differences were measured using Nei's genetic distance (D) (Nei, 1978) and illustrated in a dendrogram constructed by the unweighted pair-group method using arithmetical averages (UPGMA) (Sneath & Sokal, 1973). The biochemical differences strengthen morphological observations confirming the presence of three different allopatric species of *Sigara* in Italy.

01-186

IDENTIFICATION OF SOME *EMPOASCA* SPECIES BY MEANS OF ELECTROPHORESIS AND SCANNING ELECTRON MICROSCOPY

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DI.VA.P.R.A. - Entomologia e Zoologia applicate all'Ambiente "Carlo Vidano", Università di Torino, Grugliasco, Italy

The genus *Empoasca* Walsh is an economically important group of phytophagous leafhoppers. In Piedmont three species, *E. decipiens* Paoli, *E. solani* (Curtis) and *E. vitis* (Goethe), have been recognised and their life history and pest status are well known.

Empoasca males are classified by means of the analysis of genitalia, while females cannot be identified with certainty from their morphological characteristics. As it is desirable to identify the species also using the female sex, other features by means of electrophoresis and scanning electron microscopy have been searched.

Tetrazolium oxidase or superoxide dismutase (TO or SOD) and glucose-6 phosphate dehydrogenase (G-6PDH) enzyme systems proved to be a reliable tool for classifying the three *Empoasca* species.

Also the analysis of the ovipositor showed some useful characteristics for the specific identification. The valvulae dissected from the females used for electrophoresis have been observed and each species specific character has been associated with electrophoretic data.

01-187

MORPHOLOGICAL ELEMENTS FOR A KEY TO SUBGENERA OF THE GENUS *BOMBUS* LATREILLE S.L. REPRESENTED IN ITALY (HYMENOPTERA APOIDEA).G. Bolchi, F. Intoppa¹, M. G. Piazza.¹

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According to a list drawn up on the basis of the literature, examination of museum material and specimens captured for study purposes, there are 33 Italian species of the *Bombus* Latreille s.l. divided amongst 13 subgenera.

Of these subgenera the morphological characteristics of queen and worker heads are described, with definition of the variability and diagnostic value. The elements studied concern: the ratio of length and breadth of the head itself; the direction of the longitudinal axis of the eyes; forehead puncture; dorsal furrow of the temples; dimension, shape and puncture of the clypeus; proportions of the malar space; the position of the ocelli; dimensions of the antennal segments; the structure of the superior labrum; the structure of the mandible.

The characteristics listed, derived from the descriptions and dichotomic keys of various Authors, have been objectively compared with examples of each species, thereby permitting confirmation or correction.

Here these characteristics are set out in synoptic tables and illustrated with SEM photographs.

01-188

IDERATUS THOMSON, 1864 (COLEOPTERA, CERAMBYCIDAE, CERAMBYCINAE): REDESCRIPTION AND TAXONOMIC POSITION.

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Ideratus Thomson, 1864 was proposed for a single species, *I. cyanipennis* Thomson, 1864, from Colombia, and placed near *Callichromopsis* Chevrolat, within the Subtribe Callichromitae, Division Callichromitae Verae, far from the Compsocerini Thomson, 1864 (THOMSON, 1864). LACORDAIRE (1869) included *Callichromopsis* in Compsocerini but did not mention *Ideratus*. After the original description, the only mention to the genus was that of AURIVILLIUS (1912) who included it in Compsocerini. Therefore, *Ideratus* remained almost unknown up to now. The only known representative of *I. cyanipennis* is the type-specimen, a female.

The genus and the species are redescribed and illustrated based on the study of the holotype. The taxonomic position of *Ideratus* in relation to predominantly Neotropical tribes with finely faceted eyes is discussed. It was found that the genus is not a Compsocerini, and also shows no affinities with genera of other Neotropical tribes. So it is removed from Compsocerini and until further studies are possible, considered as in Incertae sedis.

01-189

A NEW SPECIES OF *AZYA* MULSANT FROM BRAZIL (COLEOPTERA, COCCINELLIDAE, COCCIDULINAE) FEEDING *PULVINARIA PARANAENSIS* HEMPEL, 1929 (HOMOPTERA, COCCIDAE) ON *ILEX PARAGUARIENSIS* ST.HIL. (AQUIFOLIACEAE)L.M.Almeida, R.C.Z.de Carvalho¹

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The genus *Azya* Mulsant has thirteen Neotropical species. Most of them have potential importance for biological control and have been introduced in other regions of the world. A new species from Bituruna and Cruz Machado, Parana State, was found feeding voraciously on *Pulvinaria paranensis* Hempel, 1929 from *Ilex paraguariensis* St.Hil. an important crop of southern Brazil. A detailed morphological study of the male and female genitalia is presented and illustrated. This new taxon is included into the existing key to species.

01-190

PHENETIC ANALYSIS OF BRAZILIAN SPECIES OF *AMBLYCERUS* THUNBERG, 1815 (COLEOPTERA:BRUCHIDAE).

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A detailed morphological study of Brazilian species of *Amblycerus* Thunberg, 1815 resulted in the definition of 83 characters, mainly external, from which 34 were quantitative and 49 qualitative. The phenetic relationship among the 56 OTU's was performed using both the Cluster and Principal Component Analyses. The results obtained by these two complementary technics showed two large groups, suggesting the existence of two taxa at the generic level; one of them was considered new. The Cluster Analysis, regarded as the most appropriate technique to reproduce distances between close neighbors, made possible the definition of 22 species groups with 1 to 8 species each.

01-192

STUDIES IN THE SYSTEMATICS AND BIOLOGY OF THE EASTERN PALAEARCTIC MORDELLID BEETLES (COLEOPTERA: MORDELLIDAE)

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By far, the mordellid beetles were poorly examined in the Eastern Palearctic. In 1975-1995, over 7,000 specimens were collected by sweeping and reared by the author. Numerous materials from the most important collections, including primary type specimens, were examined. This work was resulted in the revised list of taxa occurring in this area. The further studies of reared materials allow to identify several species of the genera *Tomoxia*, *Hoshihananomia*, *Variimorda*, *Mordella*, *Mordellistena* et al. from larvae.

There are significant affinities in larval morphology and habitats between several species forming groups those include both closely related and non-related species with similar biology; they are recognised as the morphofunctional groups, showing numerous cases of homoplastic characters. They appear independently due to general direction of morphofunctional specialisation in different lineages of Mordellidae. Further, the comparative morphological study of wing structures gives a unique possibility to understand the variability of the wing venation and to hypothesise the ways of their specialisation. Life habits of the Mordellidae are broadly reviewed in the presentation.

01-191

A PHYLLOGENETIC ANALYSIS OF THE *EVOPLOTUS* GROUP (HETEROPTERA, PENTATOMIDAE, PENTATOMINI)

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The *Evoplutus* group includes three neotropical genera: *Evoplutus* Amyot & Serville, *Pseudevoplutus* Ruckes and a new genus described by Grazia & Becker (in press). The monotypic genus *Evoplutus* was revised by Grazia et al. 1993; a comparison of morphological characters, including the genitalia, between this genus and *Pseudevoplutus sensu* Ruckes was presented. Grazia et al. 1994 based on detailed studies of the genitalia of both sexes proposed the breakdown of *Pseudevoplutus*. Besides the type species *P. paradoxus* Ruckes, 1958, three new species were added: *P. costalimai* Grazia et al., 1994, *P. peruvianus* Grazia et al., 1994 and *P. vittatus* Grazia et al., 1994. The remaining three species described in *Pseudevoplutus* (*P. longicornis* Ruckes, 1959, *P. casei* Thomas, 1980, and *P. mexicanus* Brailovsky & Barrera, 1982) were transferred to a new genus in which were also described two new species, one from Costa Rica and another from Venezuela (Grazia & Becker, in press). In this paper a table showing the morphological relationships between *Pseudevoplutus* and the new genus was included. Within the family Pentatomidae, just a few papers treated the relationships among genera on modern phylogenetic basis. In the article, the analysis was based in 45 characters, which polarization was obtained by comparison with the outgroup (*Mormidea* Dallas and *Arvelius* Spinola). The cladogram obtained, using HENNIG86 supported the monophyly of the group.

01-193

ON THE IMPORTANCE OF SENSILLA IN THE INTERPRETATION OF WING VEIN REDUCTION IN APHIDIINES (HYMENOPTERA BRACONIDAE)

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Aphidiines show a variety of wing venations which, starting from a braconoid type (*Ephedrus* Haliday), reach strong simplification (e.g. *Diaeretiella* Stary', *Trioxys* Haliday). Morphological, embryological and ethological data support the idea that, in this group, wing vein reduction increases with the degree of adaptation to parasitoidism. The process of fading and obliteration of venation leads to disappearance of closed cells in the distal part of the wing. In some species of the genus *Aphidius* Nees, the pigmentation of the distal tracts of some veins involved in the process of fainting is variable and causes problems in species discrimination and genus characterization. In these cases, the presence-absence of presumable placoid sensilla, which characterize definite tracts of veins can help in evaluating the degree of their reduction. The closely related parasitoids *Aphidius salicis* Haliday and *Aphidius aquilus* Mackauer can show convergences in degree of fainting of the intermedian+median and interrational veins. Nevertheless, the presence of at least one sensillum at the point where these two veins meet is distinctive of *Aphidius salicis* Haliday in which evidently this point still preserves its function. In *Aphidius aquilus* Mackauer instead, these sensilla and, presumably, the corresponding nerval termination leading to them have irreversibly disappeared. In fact *A. aquilus* is usually reported as having *Diaeretiella*-like reduction of veins. A comparative examination of several other genera of Aphidiines (*Praon* Haliday, *Lysiphlebus* Foerster, *Lipolexis* Foerster, *Monoctonia* Stary') showing different degrees of wing vein reduction has been carried out. This study confirmed the close association of wing veins with these presumed sensorial structures.

01-194

AN IDENTIFICATION EXPERT SYSTEM FOR TORTRICINAE (LEPIDOPTERA) IN CHINA BY COMPUTER IMAGE ANALYSIS OF VENATION

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The authors have stored into a computer the male and female venations of more than 250 species of tortricids in China. Anyone who wants to identify a chinese tortricid, just needs to draw a figure of its venation, input it into the computer and go through the three steps developed by the authors: 1. Localization of vien point by two-center method; 2. Model modification method; 3. Dissimilarity sequence analysis. Then, he will obtain a correct species name with an accuracy over 95%.

Besides, the authors have also stored into computer the morphological discriptions, life-history, distributions and figures of adult, male and female genitalia of these 250 species, so they can be easily called out for further checking and ensure the success up to 100%.

01-195

PHYLOGENETIC ANALYSES OF THE FAMILIES OF SHORT-TONGUED BEES (HYMENOPTERA: APIFORMES)

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Phylogenetic analyses of short-tongued bees were made using adult morphological characters of species representing 48 genera of short-tongued bees, 9 genera of long-tongued bees, and 8 genera of wasps in the superfamily Apoidea. Two series of analyses compared the phylogenetic implications of competing hypotheses of character evolution for the glossa. In each series, the effects of various character weighting schemes were compared.

The monophyly of bees is strongly supported. Short-tongued bee families found to be monophyletic in this study are Andrenidae (including Oxaeinae), Halictidae, Stenotritidae (represented in this study by only one species), and probably Colletidae. Melittidae are clearly paraphyletic, and include the short-tongued bees most closely related to long-tongued bees. The Melittidae are subdivided into three families, the Meganomiidae, Dasypodidae, and Melittidae proper. Several conflicting hypotheses of relationships among the families of short-tongued bees are supported in the various analyses, and are highly sensitive to character weighting and assumptions about polarity or ordering of transformation series for a handful of glossal characters.

01-196

PHYLOGENETIC ANALYSIS OF THE FAMILY GROUPS WITHIN THE INFRAORDER PENTATOMOMORPHA (HETEROPTERA)

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Fifty-seven characters are used to analyze cladistically 34 family group taxa in the Pentatomomorpha. The six superfamilies Aradoidea, Coreoidea, Idiostoloidea, Lygaeoidea, Pentatomoidea, and Pyrrhocoroidea are recognized. New superfamily combinations include the transfer of the Henicocorinae to Idiostoloidea, the Piesmatoidea to Lygaeoidea, and the lygaeid subfamily Psammidae to Piesmatidae. The family Lygaeidae (*sensu lato*) is shown to be paraphyletic and is separated into ten monophyletic family groups. The analysis is performed using both HENNIG86 and PAUP. Character distributions and results using weighted and unweighted data are discussed. A key is offered to facilitate recognition of the superfamilies and a revised classification of the infraorders and their subordinate taxa is proposed.

01-197

PHYLOGENY OF LARVAE AND ADULTS IN W-PALAEARCTIC ONTHOPHAGINI (COL., SCARABAEOIDEA): A TEST OF TAXONOMIC CONGRUENCE.

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Character congruence is a measure of classifications stability. Since phylogenies are not equally probable, however, not all character sets are adequate bases for inferences on organic evolution.

In order to test the consistency of phylogenies and stability of present classifications of the W-Palaearctic Onthophagini, a test of taxonomic congruence among cladograms showing phylogenetic relationships of adults and larvae was carried out. Twenty five adults characters (external morphology and male/female genitalia), and 23 larval characters were polarized. Adults of 31 species and larvae of 19 species, belonging to all Onthophagini's subgenera of the W-Palaearctic Region, were studied.

Phylogenetic trees were calculated running "Hennig86" and "PAUP" packages. Consensus trees and dissimilarity measures (Partitions, Symmetric Difference -SD-, Strict Joint Assertions -SJA-) among tree profiles were obtained by using the "COMPONENT" package.

Results indicated that SD and SJA measures for triplets among phylogenies inferred from adults and larvae range from 0.355 to 0.544 (mean=0.468), and from 0.269 to 0.477 (mean=0.377), respectively. For quartets, SD and SJA measures range from 0.540 to 0.652 (mean=0.597), and from 0.344 to 0.487 (mean=0.416). Comparisons between Nelson consensus trees of adults and larvae, show quite similar figures for all dissimilarity measures.

Differences in the number of studied taxa (adults of 31 species and larvae of 19 species) show not bias on tree dissimilarity measures.

A brief discussion on the possible causes of this moderate taxonomic incongruence between phylogenies inferred from adults and larvae is presented, however, more questions than answers still remain.

01-198

TAXONOMY AND BIOLOGY OF JAPANESE ADELIDAE (LEPIDOPTERA)

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The family Adelidae of Japan comprises 3 genera, i.e., *Adela*, *Nemophora*, *Nematopogon*. The family has been fragmentarily studied and the arrangement was made by Moriuti (1982) checking up 25 species from Japan. However, no revisional work has been made since Matsumura (1932) listed Japanese species. Recently Hirowatari (1995) added two new *Nemophora* species to the fauna.

As a result of tentative arrangement, at least 31 species were recognized in Japanese Adelidae, including 5 new species and 1 unrecorded one.

Though the adelid species is relatively abundant in Japan, life history of the almost species has been unknown except only two species, *Nemophora raddei* (Rebel) and *N. albi antennella* Issiki. In order to provoke attention, possible host plants of some species are provided mainly based on the author's observation.

In addition, daily activity patterns of some adelid moths are discussed in the light of evolution of their precopulatory behavior.

01-200

STAPHYLINIFORMIA (COLEOPTERA): A MONOPHYLETIC GROUP?
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A close relationship between the superfamilies Hydrophiloidea, Histeroidea (sometimes included in the former) and Staphylinoidea has long been recognized. The three groups are collectively referred to as Staphyliniformia. The phylogenetic status of this large series of polyphagan Coleoptera has been reanalysed on the basis of the distribution of approximately 120 adult and larval characters in 30 staphyliniform subgroups and 7 representatives of more-or-less closely related groups of beetles.

The analysis confirms the current concepts of Hydrophiloidea, Histeroidea and Staphylinoidea and strongly supports the hypothesis that the family Hydraenidae belongs to Staphylinoidea rather than to Hydrophiloidea as often assumed. But the assumption that the three superfamilies constitute a monophyletic "Staphyliniformia" is doubtful. Rather, it appears that the Scarabaeoidea, which are often considered closely related to (but not part of) "Staphyliniformia", share several derived features with Hydrophiloidea and Histeroidea, suggesting that they may be the sister group of these two superfamilies. Staphylinoidea should then be considered as the sister group of Scarabaeoidea, Hydrophiloidea and Histeroidea. Characters defining these groups and supporting hypothesis about their relationships are discussed.

01-199

SEARCHING FOR NEW SYSTEMATIC CHARACTERS IN PALAEARCTIC PACHYDEMINAE (COL., SCARABAEOIDEA)

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Studies on systematics of Pachydeminae have shown an unstable taxonomy. Such instability arises from two closely related facts: i) difficulties in establishing variability patterns (inter or intraspecific) for each character; and ii) many species are poorly represented in the collections, being often limited to the typical series.

This methodological problem is attacked in this work by reviewing the taxonomic goodness of usual characters (mostly external morphology) and by considering new ones (male genitalia). An identification of plesiomorphies and apomorphies has been attempted in all cases. Preliminary results on molecular characters (allozyme electrophoresis) have been obtained too. Until now, 6 genera and 93 species have been studied. In particular, the entire group of genera inhabiting the W-Palaearctic area (except for three monospecific genera) has been analyzed. The average taxonomic sample was 51% species/genus ranging from 30% (*Tanyproctus*) to 100% (*Ceramida*). In all, 24 external characters and 19 concerning male genitalia (genital segment, tegmen and endophallus) are discussed. The results are the following:

i) Male genitalia provides new, useful taxonomic characters and good hypothesis of homology, character states being better defined than in external morphology. Mosaicism in character states is more often found when codifying external morphology.

ii) There are more generic apomorphies (33) than genera to be systematized (14), thus allowing easy and unequivocal identification. The degree of character polymorphism, however, varies among genera determining different species diversity and taxonomical complexity, the greater the polymorphism, the easier the taxonomy.

iii) Allozyme electrophoresis of two populations belonging to different species shows genetic differences congruent with morphological and genitalia homologies.

01-201

PHYLOGENETIC ANALYSIS OF THE NORTH-TEMPERATE SUBGENERA OF *LASIOGLOSSUM* (HYMENOPTERA: HALICTIDAE) USING NUCLEOTIDE SEQUENCE DATA.

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The bee family Halictidae includes over 5000 species worldwide. Within the halictid genus *Lasioglossum* there are roughly 1000 species worldwide. Social behavior in *Lasioglossum* is highly variable among species and ranges from species in which females nest solitarily to species with highly complex forms of social behavior, involving sterile workers, morphologically differentiated queens and workers, and overlap of generations. The high degree of variability in social behavior among the species of *Lasioglossum* makes this group a model system for investigating the origins (and possibly losses) of social behavior in insects. While the monophyly of *Lasioglossum* sensu lato is supported by a number of morphological characters, such as reduced distal wing veins in females and a ventrally directed retrorse lobe in males, the relationships among the 18 currently recognized subgenera of *Lasioglossum* is unresolved. *Paralictus* almost certainly makes *Dialictus* paraphyletic, *Paralictus* + *Dialictus* likely arise from within *Evyllaes*, and *Lasioglossum* sensu stricto may be paraphyletic with respect to *Dialictus* + *Evyllaes*. In order to resolve the relationships within *Lasioglossum*, I have begun generating a preliminary data set based on approximately 1000 bps of Elongation Factor 1- α , a nuclear gene that has a number of desirable traits for higher-level phylogenetic analysis, including an unbiased nucleotide composition, a slow rate of nucleotide substitution, and a low level of homoplasy in phylogenetic analysis. In this talk I will present a preliminary phylogeny for the north-temperate subgenera, including *Lasioglossum* sensu stricto, *Dialictus*, *Evyllaes*, *Sphecodogastra*, *Paralictus* and *Hemihalictus*. Australian, African and Asian subgenera will be added later as more specimens become available.

01-202

THE EVOLUTION OF HABITAT USE AND DIET IN THE SCARABAEOIDEA: A PHYLOGENETIC APPROACH

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The evolution of habitat use and of diet in the Scarabaeoidea is objectively assessed using character optimization based on a phylogenetic analysis of the superfamily. The ancestral habitat was clearly soil; larval food was microbial- (mainly fungus-) enriched humus; adult food was fungi. The evolution of the major clades is supported by the fossil record. The first major evolutionary event was after the Triassic mass extinction (200 MY) with radiation of the families currently considered to be "primitive" in the Jurassic (180-160 MY). The second major radiation was after the Cretaceous-Tertiary mass extinction (65 MY) when the taxa considered to be "higher" scarabaeoids diversified. The primitive clade is far less diverse than the more modern one. Extinction of taxa in the older clade made way for the diversification of the more modern scarabaeoid groups which was facilitated by the opening of two "new" adaptive zones in the form of the angiosperms and artiodactyl dung. The same themes in the evolution of habitat use and diet are repeatedly played out in both major clades, despite the differences in the floras and faunas of the Mesozoic and the Cenozoic. The phylogenetic tree appears balanced, with respect both to its topology and the ecological characters that are mapped onto it since the clades are virtually mirror images of each other (i.e., similar patterns of habitat use and diet have evolved in the post-Triassic, Mesozoic clade, and the post-Cretaceous, Cenozoic one).

01-204

STUDIES IN THE SYSTEMATICS AND BIOLOGY OF THE EASTERN PALAEARCTIC MORDELLID BEETLES (COLEOPTERA: MORDELLIDAE)

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By far, the mordellid beetles were poorly examined in the Eastern Palearctic. In 1975-1995, over 7,000 specimens were collected by sweeping and reared by the author. Numerous materials from the most important collections, including primary type specimens, were examined. This work resulted in the revised list of taxa occurring in this area. The further studies of reared materials allow to identify several species of the genera *Tomoxia*, *Hoshihananomia*, *Variimorda*, *Mordella*, *Mordellistena* et al. from larvae.

There are significant affinities in larval morphology and habitats between several species forming groups those include both closely related and non-related species with similar biology; they are recognised as the morphofunctional groups, showing numerous cases of homoplastic characters. They appear independently due to general direction of morphofunctional specialisation in different lineages of Mordellidae. Further, the comparative morphological study of wing structures gives a unique possibility to understand the variability of the wing venation and to hypothesise the ways of their specialisation. Life habits of the Mordellidae are broadly reviewed in the presentation.

01-203

THREE NEW ASIAN GENERA OF FIRE-COLORED BEETLES (COLEOPTERA:PYROCHROIDAE:PYROCHROINAE)

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When present, cranial pits in adult male pyrochroines may be located behind the eyes, as in *Schizotus*, or between the eyes. In the latter, the pit may consist of a single, shallow impression--as in the European *Pyrochroa* and Asian *Eupyrochroa*--or pits may be well developed and paired as in the North American *Neopyrochroa* and the Asian *Pseudopyrochroa*. **New Genus "A,"** is required for *Pseudopyrochroa sumatrensis* (Pic) (Indonesia, Malaysia-Sabah) + *P. nigripennis* Pic (peninsular Malaysia) + *P. atricolor* Pic (peninsular Malaysia). In this unique assemblage, cranial pits are completely lacking, and the parameres of the male genitalia are very abruptly and widely separated distally.

The monophyly of *Pseudopyrochroa* + **New Genus "B"** may be hypothesized by synapomorphies associated with the external male genitalia. In both taxa, the dorsolateral apices of the parameres are bilaterally toothed, with each tooth projecting basally. The apex of the penis is also provided with a dorsomesal, basally recurved hook. **New Genus "B"** is required for the Vietnamese *Pseudopyrochroa binhana* Pic and several related species presently assigned to *Pseudodendroides*. The structure of the male genitalia precludes placement of these species in *Pseudodendroides*. In *Pseudodendroides* the parameres of the male genitalia are widely separated, distally. This character represents a probable synapomorphy also exhibited by *Neopyrochroa* and **New Genus "C"** (based upon an undescribed species from the Darjeeling District of India).

01-205

SYSTEMATICS OF THE AUSTRALIAN NASUTITERMITINAE (ISOPTERA: TERMITIDAE)

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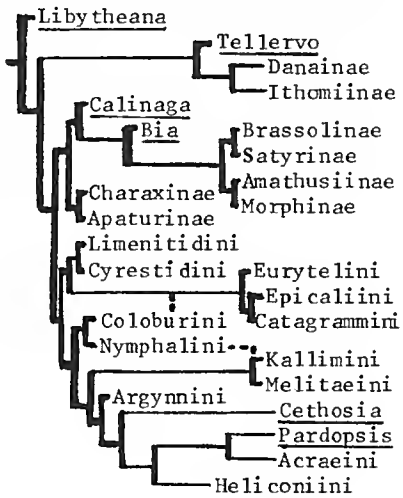
The named species of Australian Nasutitermitinae are currently placed in six genera. The majority of species are distributed between the genera *Nasutitermes* (17 species), and *Tumulitermes* (17 species). This division is unsatisfactory in that different castes of some species may be placed in different genera when considered alone.

A study of external morphology, patterns of mandibular dentition and gut structure indicates that the 34 species belong to at least four genera. This study also reveals that the group is particularly diverse in Australia's arid region, with approximately 40 species awaiting description.

01-206

SYSTEMATICS AND PHYLOGENY OF NYMPHALID BUTTERFLIES (SENSU LATU) BASED ON JUVENILES, WITH AN EXPERIMENTAL STUDY OF THE EVOLUTION OF HOST PLANT PREFERENCE
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Nymphalid butterflies are cosmopolitan, comprising about 6000 described species, strongly differentiated into at least five groups morphologically recognizable in all stages, with adults further divergent into about twenty additional groups. The relationships among these groups were investigated through 250 characters drawn mostly from immatures, with an emphasis on Neotropical species (74); 20 Old World species, in isolated or closely related groups, were added to further highlight the divisions and associations. *Libytheana* was initially used as an outgroup, with plesiomorphic character states also checked against other butterfly groups; 11 apomorphies then defined the Libytheinae. The matrix was processed with Hennig86, PAUP, and MacClade with the inclusion of an all-0 hypothetical ancestor. The resulting phylogeny (right) was used to investigate the hypothesis that species are more likely to accept foodplants used by members of more basal branches than those used in branches apical to them. The results in general supported this assumption, especially at the tribal level, but also at the subfamilial level for widely used plant families like Urticaceae, Ulmaceae, Violaceae, Moraceae and Fabaceae. Oviposition mistakes by females also gave clues about possible paths for colonization of new hosts.



01-208

A SYSTEM OF THE LASIOCAMPIDAE (LEPIDOPTERA) OF HOLARCTIC FAUNA

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A suprageneric system is proposed for the Lasiocampidae of the holarctic fauna. Type-genera are underlined.

- 1. *CHONDROSTEGINAE* Tutt, 1902.
 - 1.1. *Chondrostegini* Tutt, 1902 - *Chondrostega* Led.
- 2. *MALACOSOMINAE* Tutt, 1902.
 - 2.1. *Malacosomini* Tutt, 1902 - *Malacosoma* Hbn.
- 3. *LASIOCAMPINAE* Harris, 1841.
 - 3.1. *Lachneini* Tutt, 1902 - *Eriogaster* Germ.
 - 3.2. *Lasiocampini* Harris, 1841 - *Lasiocampa* Schrank, *Amurilla* Auriv., *Ergolea* Dun., *Cerberolebeda* Zolt.
 - 3.3. *Macrothylaciini* Tutt, 1902 - *Macrothylacia* Ramb.
- 4. *POECILOCAMPINAE* Tutt, 1902.
 - 4.1. *Poecilocampini* Tutt, 1902 - *Poecilocampa* Steph., *Trichiura* Steph., *Hypopacha* Newmoegen & Dyar.
- 5. *MACROMPHALIINAE* Franclemont, 1973.
 - 5.1. *Macromphaliini* Franc., 1973 - *Macromphalia* Felder, *Tolyte* Hbn., *Apotolyte* Franc.
- 6. *PINARINAE* Kirby, 1892.
 - 6.1. *Selenepherini* Tutt, 1902 - *Cosmotriche* Hbn., *Euthrix* Meigen, *Cosmoptera* Laj., *Eteinopla* Laj., *Chilena* Wlk., *Sena* Wlk., *Beralade* Wlk., *Psilogaster* Wlk., *Lenodora* Moore, *Micropacha* Roepke.
 - 6.2. *Trabalini* Tutt, 1902 - *Trabala* Wlk., *Crinocraspeda* Hamps.
 - 6.3. *Pinarini* Kirby, 1892 - *Pinara* Wlk., *Streblote* Hbn., *Stoermeriana* de Fr. & Witt, *Lebeda* Swinh., *Paralebeda* Auriv., *Bhima* Moore, *Suana* Wlk., *Pachypasa* Wlk., *Metanastria* Hbn., *Kunugia* Nag., *Pachypasoides* Mats., *Dendrolimus* Germ., *Euwachyptera* Barnes & McDunn., *Caloecia* Barnes & McDunn.
 - 6.4. *Gastropachini* Newmoegen & Dyar, 1893 - *Gastropacha* Ochs., *Phylloidesma* Hbn., *Heteropacha* Harv., *Paradoxopla* Laj.
 - 6.5. *Odonestidi* Tutt, 1902 - *Odonestis* Germ., *Takanea* Nag., *Radhica* Moore, *Arguda* Moore, *Bhareta* Moore, *Syrastrena* Moore, *Syrarstrenopsis* Gruenb., *Odontocraspis* Swinh., *Hallicornia* Hbn.

01-207

MORPHOLOGY OF THE PROBOSCIS OF BUTTERFLIES (LEPIDOPTERA: RHOPALOCERA)

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The extremely elongated proboscis of butterflies has been described only in a few species so far. No comprehensive comparison of the morphology of the proboscis exists and there is no attempt to evaluate the features in functional and evolutionary context.

Using light and scanning electron microscopy, the present investigation of approximately 100 species of butterflies compares the proboscis wall and its surface structures, the inflow-slits-forming structures at the tip, and the form and distribution of the different types of sensilla. The evaluation of the transformation series and the polarity of these features allow the reconstruction of the plesiomorphic character state of the proboscis in Papilionoidea. Divergent evolutionary trends led to distinct combinations of features which characterize the probosces of Papilionidae, Pieridae, and Lycaenidae. Special attention is given to the highly divers probosces of Nymphalidae (sensu lato) and their correlations with specialized feeding habits. The systematical relationships of the subfamilies in Nymphalidae are discussed using proboscis features in addition.

These results contribute to systematical considerations on higher taxonomical level and provide the basis for a better understanding of the functional role of the different structures on the proboscis during flower visiting.

01-209

MICROPYLAR STRUCTURE OF THE EGGS OF SOME SOUTH AFRICAN HEPIALOID MOTHS (LEPIDOPTERA: HEPIALIDAE, PROTOTHEORIDAE)

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The micropylar area situated at the anterior pole of the lepidopteron egg contains a variable number of micropyles permitting the passage of spermatozoa into the egg. The micropylar area forms part of the chorion and due to its variable nature may be of use as a taxonomic character. The number of micropyles may also be a diagnostic feature. Eggs of four species of hepialoid moths were collected in the Western Cape. Scanning electron microscopic studies indicate distinctive and varying structure of the egg surface and micropylar area of Hepialidae and of Prototheoridae. The surface sculpture of the eggs differ, consisting of small hemispherical protuberances in the Hepialidae and barlike blunt spicules in the Prototheoridae. Micropylar areas were distinctive, differing between the two families, being rounded to subtriangular and consisting of an elevated frame with variable radiating ridges in Hepialidae or only an elevated rim in Prototheoridae. The swollen inner margin encircled a smooth area, two or three micropyles situated equidistant under the inner margin of the frame. The number of micropyles were usually two but in many instances eggs with three micropyles were also present. Studies elsewhere indicate that all hepialoid eggs examined so far only possess two micropyles.

01-210

TEMPERATURE-INDUCED POLYPHENISM IN AFRICAN *BICYCLUS* BUTTERFLIES (LEPIDOPTERA: NYMPHALIDAE): ADAPTATION OR GHOST OF THE PAST?

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Temperature-induced variation and norms of reaction have been analyzed for wing pattern elements of six *Bicyclus* species using multivariate statistical techniques. A phylogenetic reconstruction based on adaptive plastic wing characters was compared with a cladogram built on 'non-adaptive' characters.

Results demonstrate that:

(1) Different wing pattern characters are under different control: 'exposed' characters of butterflies at rest position are highly sensitive to temperature variation, whereas 'hidden' characters, only visible during active behaviour, are dominated by species differences. In general the sensitivity of the former can be attributed to their proposed function in deflecting predators.

(2) Phylogenetic distances are to some extent reflected in ordination in both PCA- and DFA-space: closely related species remain close in both ordinations. The more distantly related species differ in ordination from a pattern as suggested by a phylogenetic reconstruction. It is argued that the wing pattern variation of these species reflects both adaptive processes and historical relationships.

01-212

THE PHYLOGENY OF BLATTARIA AND MANTODEA BASED ON MALE GENITAL MORPHOLOGY

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The male genital organs (phallomeres) of Blattaria and Mantodea are very complex structures supplied with many sclerites and muscles. They are always completely asymmetrical. In Isoptera, the third Dictyopteran order, phallomeres are missing.

By a detailed morphological analysis of the sclerites, muscles and formative elements (in- and evaginations of the cuticular surface) a homology concept for the phallomeres of 4 species of Mantodea and 10 species of Blattaria could be elaborated, and a common ground plan of Blattaria and Mantodea could be reconstructed. Asymmetry is homologous in both orders, and the pattern of phallomere morphology is very similar - in gross structure as well as in many details.

In Mantodea the basal dichotomy is between Mantoididae (not Chaeteessidae) and the other families, in Blattaria it is between Blattidae and the other families. The remaining Blattaria are divided into three groups containing: (1) Tryonicinae (previously assigned to Blattidae); (2) Cryptocercidae, Polyphaginae and Lamproblattinae (the latter previously assigned to Blattidae); (3) Blattellidae and Blaberidae.

Within group (3) Blattellidae are paraphyletic with regard to Blaberidae: Anaplectinae are the basal off-shoot, followed by several branches of Plectopterinae, which are a paraphyletic taxon, too. The higher Blattellidae (Blattellinae, Ectobiinae, Nyctiborinae) and Blaberidae together are a monophyletic group. The phallomeres of Blaberidae and "Plectopterinae" are mirror-images of the phallomeres of other Blattellidae. The hypothesis of Bohn 1987 that this is due to a change of left-right-asymmetry - and not parallel evolution - could be affirmed: For example, *Blaberus* (Blaberidae, phallomeres reversed) is nearly identical to *Nyctibora* (Nyctiborinae, phallomeres normally orientated) in phallomere morphology.

01-211

TAXONOMY AND DISTRIBUTION OF THE GENUS *CALLIPTAMUS* AUD.-SERV. (ORTHOPTERA: ACRIDIDAE)

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Taxonomic composition of the genus *Calliptamus* was described by Jago (1963). The phenotypic and morphological analyses permit us to restore a generic status of the *Metromerus* group and to discuss a status of some forms [*C. (italicus) reductus*, *C. (barbarus) nanus*, *M. (coelestriensis) hissaricus*].

The generic range occupies the territory from Spain to the Far East and from North Poland to the Arabian Peninsula. The centers of species diversity of this genus can be observed in the western part of its range.

Some species of this genus (*C. italicus*, *C. barbarus*, *C. turanicus*, *C. abbreviatus*) are the abundant forms in the southern steppes, the semi-deserts and the deserts. Their outbreaks were described for the Mediterranean and Saharan-Gobian regions.

We analyzed the range structures and the local population distribution of these species over biogeographical regions, landscapes and their units. This analysis allows us to develop an idea that every part of a range is characterized by a specific type of dynamics and migrations.

01-213

PHYLOGENETIC RELATIONSHIPS AMONG THE GENERA OF THE JAPANESE HELEOMYZIDAE (DIPTERA)

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The Japanese family Heleomyzidae consists of 2 subfamilies (9 genera).

A single genus *Suillia* Robineau-Desvoidy belongs to Suilliinae and the other 8 genera, *Aecothea* Haliday, *Eccoptomera* Loew, *Heleomyza* Fallén, *Morpholeria* Garrett, *Orbellia* R.-D., *Schroederella* Enderlein, *Scoliocentra* Loew and *Tephrochlamys* Loew to Heleomyzinae.

Twelve morphological characters were used in the phylogenetic analysis to reconstruct the relationships among Japanese 9 genera of Heleomyzidae.

Suillia (Subfam. Suilliinae) has mostly modified characters and is more specialized than the other 8 genera of Heleomyzidae.

Among Heleomyzinae, *Tephrochlamys* is more specialized than the rest of genera by the characters of dorsocentral bristles and apicoventral bristles on mid tibia. The other 7 genera, are segregated into 2 branches, *Orbellia* and a group of 6 genera. *Orbellia* has more plesiomorphic characters. In 6 genera, *Heleomyza* and *Scoliocentra* has more plesiomorphic characters. *Aecothea* has more specialized than the 3 genera, *Eccoptomera*, *Morpholeria* and *Schroederella*. These 3 genera are divided into *Eccoptomera* and *Morpholeria* + *Schroederella*, by the chaetotaxy of mid femur. This character state is considered to be plesiomorphic in *Eccoptomera*. The latter 2 genera have synapomorphy in this character.

01-214

BIOCHEMICAL SYSTEMATICS OF THE *PHYLLOTRETA TETRASTIGMA* GROUP OF SIBLING SPECIES (COLEOPTERA : CHRYSOMELIDAE).

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P. dilatata Thomson, 1866, *P. flexuosa* (Illiger, 1794) *P. ochripes* (Curtis, 1837) and *P. tetrastigma* (Comolli, 1859) are four closely related and morphologically very similar (sibling) species. All prefer humid biotopes, live on aquatic Brassicaceae, but differ in host plant choice.

In total 13 populations representing the four species were analysed using allozyme electrophoresis of 10 variable loci. For pairwise comparison of populations Nei (1978) unbiased genetic distance and Rogers modified genetic distance (Wright, 1978) were calculated. Trees were constructed using UPGMA and Distance-Wagner methods.

P. ochripes is a clearly distinguished species, *P. dilatata* is separated from *P. flexuosa*/*P. tetrastigma* at the level of sibling species, *P. flexuosa* and *P. tetrastigma* are separated at the level of subspecies. Host plant seem to be important in speciation within this group of species. Our results indicate that host plant shifts followed by genetic differentiation probably are the first steps in speciation in the genus *Phyllotreta*.

01-216

A NEW ARRANGEMENT OF THE GENUS *OTIORHYNCHUS* (COLEOPTERA: CURCULIONIDAE)

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Otiiorhynchus presently comprises some 1500 species; their number, however, is presumably much greater. The genus is diffused in the whole central and western Palaearctic, ranging eastwards to Altai Mountains, some species are found in northern Afghanistan, northern India and Nepal. Taxonomy of this huge genus is still confused. According to the art. 11 of the Code, names of the "Artengruppen" proposed by Reitter in 1912 and 1913 must be considered subgenera, although writers have hitherto neglected this rule. The present contribution, which applies the rules of the Code about subgenera of *Otiiorhynchus*, is meant as a first step towards a complete rearrangement of the genus. Following the proposal by Arnoldi, published in 1975 and overlooked by subsequent authors, morphology of *Otiiorhynchus* allow it to be divided in three major groups: the first includes those species with 12 or 13 elytral striae (subgenus *Dodecastichus*), the second comprises species with epipleura straight or only faintly curved outwards at the level of hind coxae (almost all species of the current subgenus *Cryphiphorus*, and those of *Arammichnus* and *Tournieria* in broad sense); the third consists of *Otiiorhynchus* with epipleura strongly curved outwards at the level of hind coxae (nearly all species of subgenera *Limatogaster*, *Tyloderes* and *Cirrorrhynchus*, and those of *Dorymerus* and *Oliiorhynchus* in broad sense). The present study does not support *Trogloorhynchus* be maintained separate from *Otiiorhynchus*: species of *Trogloorhynchus* must be placed in various subgenera. Adults of thermophilous species of *Otiiorhynchus* are found in arid zones under stones, mesophilous and relatively oligothermic ones can also be met under stones, mostly in mountain areas; several mostly criophilous species are related with forests, and can be found on trees and shrubs.

01-2015

COMPARISON OF BIOLOGICAL CHARACTERS BETWEEN TWO KARYOTYPES IN THE *CHRYSOLINA AURICHALCEA* (MANNERHEIM) COMPLEX (COLEOPTERA:CHRYSOMELIDAE).

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The *Chrysolina aurichalcea* (Mannerheim) complex is distributed widely in the Palaearctic region and in the species complex, six karyotypes have been already reported. In the Japanese Archipelago, two types of them are widely distributed, although five karyotypes have been reported. In spite of large difference (the 31 type: 2n=31.32 fundamental number=52; the 41 type=41,42 f.n.=70) in karyotype between the two, their biological characters are very similar (e.g. morphological characteristic). To analyze their relationship, further comparison of their biological characters between them was conducted from various view points.

The total complemental length of the two types were compared using their hybrid. The one in the 31 type was almost the same or slightly larger than that of the 41 type. Their developmental zero was quite similar (7.2°C and 6.7°C) but thermal constant was significantly larger (c.a. 40 day-degrees) in the 41 type(c.a. 520 day-degrees from first instar to adult). Their daily rhythm in non-reproductive adult stage was rather different. At the boundary area of both types, the habitat segregation was observed with reference to the plant cover. The seasonal cycles of both types around there were nearly the same but the reproductive season of the 41 type was found to be slightly earlier than that of 31 type.

Based on these findings and other recent studies, I discuss their interrelationships among the types and their evolution.

01-217

DIFFERENT ASPECTS OF SEXUAL DIMORPHISM IN THE COLEOPTERA BRENTIDAE

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In some brentids sexual dimorphism is absent or very slight, such as in most Cyphagoga Kolbe and Trachelizinae Lacordaire, but it is usually not only obvious but occasionally even striking, affecting various parts of the body: the head (rostrum, mandibles, antennae), pronotum, elytra, legs (femora, tibiae, tarsi), and underside (abdominal plate, last abdominal segment).

The rostrum is usually cylindrical and filiform in the females; in the males it is usually longer (*Brentus* F. spp.) and can be either very dilated apically (*Baryrhynchus* Lacordaire, *Paryphobrenthus* Kolbe), saw-toothed laterally (*Stratiorrhina* Pascoe), with a medial upright protuberance (*Hopliterhynchus* Senna) or markedly pubescent (*Lasiiorhynchus barbicornis* F.).

The male mandibles can be very robust (*Estenorhinus* Lacordaire, *Gyalostoma* Kleine), or long and falcate (*Orfilaia* Haedo Rossi). In male *Bolbocranius czikii* (Bolkay) and *B. distortus* (Westwood) the left mandible is extremely long, flat and sinuate, the right one very small. The use of such an unusual structure is unknown.

The antennae are sometimes much longer in the males, as in *Cerobates* subgen. *Jonthocerus* Lacordaire. In this subgenus the eyes of the males cover almost the entire head. In *Allagopus* Gahan the antennal articles are normally shaped in the males but enlarged and modified in the females; it is one of the rare instances in which the female, and not the male, presents strong structural modifications.

The pronotum is long, slender and narrowed in the middle in male *Brentus anchorago* L., shorter and trapezoidal in the females.

In some species the male elytra have long caudal appendages, short or absent in the females (*Ceocephalus* Guérin-Méneville and in *Phocylides* Pascoe).

The legs of the males often have more or less stout spines on the femora, and sometimes also on the protibiae (*Spatherhinus* Power). In *Cormopus* Kolbe the posterior legs are strongly modified, and differ in both sexes.

The male underside usually has a more or less concave abdominal plate, flat in the females, and a differently shaped last abdominal segment.

Both the function of the structures linked to sexual dimorphism and the use of such structures in understanding the phylogenetic relationships among genera and species are far from clear.

01-218

THE SPERM STRUCTURE OF NEMONYCHIDAE AND URODONTIDAE (CURCULIONOIDEA)

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Nemonychidae and Urodontidae are plesiomorphic families related to Curculionidae (Thompson, 1992, Kuschel, 1995). By using fixative containing tannic acid (Dallai and Afzelius, 1990) their sperm structure reveals features which fit with the general organization of Curculionidae spermatozoa (Burrini *et al.*, 1988). *Nemonyx lepturoides* shows a trilayered axoneme, a cylindrical nucleus with condensed chromatin material, a centriole surrounded by a centriolar adjunct, an axoneme of 9+9+2 type with accessory microtubules made of 16 protofilaments in their wall and with a central dense axis, two equally sized accessory bodies, one of which connected to a large "puff", two mitochondrial derivatives, which are of unequal size and of which the larger one is fully crystallized and prolonged within a posterior nuclear cavity, a dense thin layer beneath the plasma membrane at the tail end. *Urodon rufipes* shows a sperm structure similar to that described for *Nemonyx*. The main difference deals with the shape of the two accessory bodies. Sperm structure indicates that Curculionoidea have a sperm structure that is maintained throughout the whole group with only small variations in some families. It is confirmed that *Neocoenorhinus aequatus* (Rhynchitidae) spermatozoa have acquired a 9+9+0 axoneme.

The sperm model of Curculionoidea shows synapomorphies with that observed in the related superfamily Chrysomeloidea.

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01-220

COMPARATIVE MORPHOLOGY OF THE MOUTH PARTS OF THE SUPERFAMILY CURCULIONOIDEA (COLEOPTERA) AND THEIR RELATIONSHIP TO CLASSIFICATION

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The group of Coleoptera known as the superfamily Curculionoidea or weevils is of world wide distribution and includes some 60,000 described species which are contained in nine well-defined families.

The most obvious character of the Curculionoides is the prolongation of the head into rostrum, of which the functional significance may fundamentally be the adaptation for drilling the oviposition hole. Ting (1936) and Morimoto (1963) clarified the fundamental structures of the mouth organs, but they discussed with one by one for each part by dissection and not on the compound structures in situ.

Present study is intended to observe the complicated mouth organs in the normal position by SEM and partly under the binocular microscope, and to infer their possible mechanisms of feeding and relationship to classification.

01-219

PHYLOGENY AND HIGHER CLASSIFICATION OF THE WEEVILS OF THE SUBFAMILY RAMPHINAE-COMPLEX (COLEOPTERA, CURCULIONIDAE)

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The weevil subfamily Ramphinae-complex includes following tribes (or subfamilies): Anthonomini, Tychiini, Ochyromerini, Ellescini, Demimaeini, Acalyptini, Derelomini, Ramphini, Dinorhopalini, Anoplini, Eugnomini, Lignyodini and Prionomerini. The definitions of these taxa are various among taxonomists by the lack of conspicuous features. Therefore, the complex is one of the most problematical taxa among the Curculionidae. Although the members of the complex are considered to be closely related to each other, their exact phylogenetic relationships have not been inferred yet.

Present study is aimed to propose a new system of the higher classification based on the inferred relationship from the Asian materials.

01-221

THE PALEARCTIC PERITELINI: THE PRESENT KNOWLEDGE (COLEOPTERA, CURCULIONIDAE, POLYDRUSINAE)

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The present knowledge concerning *Peritelini* (Coleoptera, Curculionidae, Polydrusinae), on the faunistic and taxonomical point of view as result of a nine years long field researches are synthetically exposed. Totally 30.000 samples were examined (about 18.000 of them collected by the Authors in all west mediterranean countries) and arranged in 11 genera and 200 species (unpublished included). For each genus the comprehensive number of recognized species and for each country, the *Peritelini*'s number till now described, is reported. Biological, ecological, zoogeographical notes and drawings of the endophallic structures are also added.

01-222

PRESENCE OF STREPSIPTERA LARVAE IN POLLEN COLLECTED BY *APIS MELLIFERA* L. (STREPSIPTERA - HYMENOPTERA: APOIDEA).

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Strepsiptera first instar larvae are dispersed in the environment and often settle on plants and particularly on flowers where they wait for a host to parasitize. So they may be picked up by workers of *Apis mellifera* L. collecting nectar and pollen from the flowers and incorporated in the pollen loads together with the pollen itself. This is what we have discovered through an examination of samples of pollen taken from hives situated in several localities in the central Italian Alps. The samples were taken by means of special traps, which were inspected weekly from mid-April to mid-September over two consecutive years. The samples thereby obtained were subjected to a filth-test using analytical method especially devised for the purpose, which permitted the extraction of the Strepsiptera larvae from the pollen loads. Most of the samples proved to contain these insects, varying in number according to the season, as shown in the tables here presented. In no case did we observe stylopized bees: this would suggest that the presence of Strepsiptera larvae in the pollen loads is merely a casual consequence of the bees' antophilous behaviour.

01-224

MEDITERRANEAN COLEOPTERA CURCULIONOIDEA WITH SOUTHERN AFRICAN AFFINITIES

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Some Curculionoidea from the Mediterranean, seemingly evolved in this area, show affinities with taxa distributed in Southern Africa. Among these weevils the number of Southern African species is much larger than that of Mediterranean ones. Genera common to both areas are very few, and no native species are distributed in both regions. There is a low number of genera endemic of Mediterranean, and having Southern African affinities: most of them comprise one or few species, while related genera from Southern Africa usually consist of a larger number of species. Whereas Mediterranean weevils with Paleotropical affinities are more commonly found along the southern border of Palaearctic arid belt, their number is very low in the Pannonic-Sarmatian steppic areas. It can be reminded that some of them can be evenly found in Central Asian arid zones, some also ranging to Pakistan. These weevils apparently preserved similar ways of life as those of their supposed afrotropical ancestors. Apterous or brachypterous Mediterranean Curculionoidea having Paleotropical affinities, which are in addition mostly breeding on hypogeous parts of plants, are generally rather different from Southern African ones. This can be explained by an ancient settlement, and supported by their present relict distribution. Species developing on epigeal parts are usually winged, and in fact they are often differentiated only at specific level, this leading to suppose a more recent diffusion. It is worthy of note that all Aglycideridae, Brachyceridae, and Brentinae (sensu Thompson 1992) from Palaearctic have Paleotropical affinities.

01-223

'WEEVIL SEED PREDATORS OF DIPTEROCARPACEAE'

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Several genera of Curculionoidea (weevils) include species which feed on seeds of the economically and ecologically important tropical trees Dipterocarpaceae. These beetles can destroy up to 100% of the seeds, and may have been the driving force behind the evolution of mast fruiting of the dipterocarps in South-east Asia. The most speciose genus is *Alcidodes* (Coleoptera: Curculionidae), species of which are pre-dispersal seed predators of several dipterocarp genera. Each species is restricted to a single genus of host, although may be found on many species. Conversely, any one host species may support several *Alcidodes* species.

01-225

INFLUENCE OF *STICHOTREMA DALLATORREANUM* HOFENEDER ON PERFORMANCE OF A TETTIGONIID PEST OF OIL PALM, *SEGESTIDEA NOVAEGUINEAE* (BRANCSIK) (ORTHOPTERA:TETTIGONIIDAE).
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1. Possibility of using *Stichotrema* as biocontrol for *Segestidea* sp., major pest of oil palm, *Elaeis guineensis* in Papua New Guinea (PNG). The strepsipteran endoparasite appears to act as an effective agent in mainland PNG but is absent from West New Britain Province.
2. First stage in establishing whether use of *Stichotrema* as biocontrol agent is feasible is to ascertain the impact of *Stichotrema* on host performance.
3. Poster reports results of work correlating various variables, including gut filling, reproductive development, composition and size of the carcass with the numbers of *Stichotrema* present in field caught sexavae.
4. Results suggest that the presence of *Stichotrema* reduces host feeding and reproductive performances.

01-226

IMMATURE STAGES OF BUPRESTIDAE (COLEOPTERA) - our present knowledge and morphological characteristic.

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With respect to the fact that Buprestids are primary as well as secondary pests and also vectors of tracheomycoses, the attention that has been yet paid to larval morphology and biology of Buprestidae can be considered as minimal.

We can estimate the world Buprestid fauna to be about 12000 species but only larvae of 310 species have been described so far (2.6%). Moreover a lot of old descriptions are inapplicable for taxonomical studies. While nearly 50% of Buprestid larvae from Europe have been described, we know only 2 larvae from the Afrotropical region and 22 from the Oriental region.

From the morphological viewpoint we can recognise 4 morphoecological types: Julodis-type, Buprestis-type, Agrilus-type and Trachys-type. On the basis of larval morphology we can see that some groups are quite artificial (e.g. Trachyinae) and they call for a revision.

01-228

THE LARVAL CHARACTERS OF BUPRESTID BEETLES AND THEIR TAXONOMICAL VALUE (COLEOPTERA: BUPRESTIDAE)

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The application of larval characters in buprestid systematics is considerably limited by the insufficient knowledge of larval stages as well as by the wide-spread opinion that the evolutionary trends do not coincide on the preimaginal and imaginal stages of ontogeny, and that the larval features are mostly adaptive ones.

Comparative morphological examination has revealed the presence of two groups of larval characters: 1) inadaptive, and 2) adaptive. Inadaptive features are basically determined by the common ancestry and remain nearly constant for the main phylogenetic lineages of buprestids. Adaptive characters are closely related to biological specialization and characterize the morpho-ecological forms of buprestid larvae, which may be closely similar in systematically unrelated taxa. Several related morpho-ecological larval forms can be found within the same genus (for example, *Acmaeoderella*); as this takes place the larvae belonging to different morpho-ecological forms within the same genus are often distinguished much more clearly than the larvae from closely related genera, attributed to the same forms. There are no rigid boundaries between both character categories: the morphoclines of adaptive characters allow to elucidate the evolutionary trends and to clarify the relationship level of different taxa.

The necessity of detailed analysis and evaluation of larval characters and the possibilities of their application to buprestid systematics can be illustrated by the example of some larvae from different phylogenetic lineages which are attributed to the same morpho-ecological forms: leaf miners *Paratrachys* (*Polycestinae*) and *Trachys* (*Trachyinae*), as well as *Pterobothris* (*Buprestinae*) and *Agrilus* (*Agrilinae*).

01-227

PHYLOGENETIC ANALYSIS OF THE "PLEASING FUNGUS BEETLES" (EROTYLIDAE) BASED ON LARVAL MORPHOLOGY.

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A phylogenetic study of the "Pleasing Fungus Beetles" (Coleoptera: Erotylidae) is presented. The analysis includes 44 erotylid species (representing all five subfamilies) and eight species of the closely related families Languriidae and Cryptophagidae.

The 85 characters used in the study are derived entirely from larval morphology. Despite unique problems posed by this data source, the study finds that larvae represent a rich source of information about phylogenetic relationships in Erotylidae.

Implications of the cladogram for the taxonomy of Erotylidae are discussed. Various aspects of erotylid biology are interpreted within the proposed phylogenetic framework, including pupation behavior and fungus host preference.

01-229

TAXONOMY OF "TRILOBITE LARVAE" GENUS *DULITICOLA* MJÖBERG, 1925 (COLEOPTERA:LYCIDAE) FROM SOUTH-EAST ASIA

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"Trilobite larvae" are the neotenous females of a unique group of Lycidae. Extreme sexual dimorphism is exhibited in that the males are typical lycids while the females retain larval features and grow to many times the size of the males.

Of the three described taxa, only one i.e. *Duliticola paradoxa* from Sarawak, described by E. Mjöberg in 1925, is well known. New species from Peninsular Malaysia and southern Thailand have been described recently. A possible synonymy between *Duliticola* and *Platerodrilus* Pic, 1921 will be discussed. A classification system with more emphasis on female characters will be proposed.

01-230

FIRE-COLORED BEETLE LARVAE OF THE WORLD
(COLEOPTERA:PYROCHROIDAE:PYROCHROINAE):
ANATOMICAL AND MICROHABITAT DIVERSITY

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Pyrochroine larvae may be characterized by the presence of a well developed mandibular mola on each of the asymmetrical mandibles, a continuous arch of asperities associated with the anterior margin of the ninth abdominal sternite, and paired urogomphal pits between well developed urogomphi at the distal end of the ninth abdominal tergite. The configuration of the urogomphal plate, including the urogomphi, and urogomphal lip and the urogomphal pits, has yielded a number of important taxonomic features for elucidating relationships at and above the species level.

Larvae are typically associated with mesic conditions beneath slightly loosened bark and to some extent within decaying wood of trees. Although they have been observed to exhibit cannibalism under crowded conditions, they are not normally predaceous, and fungi appear to play a significant role in their diet. In North America, and probably elsewhere, species occupy rather specific microhabitats, thereby partitioning the dead log community and presumably minimizing competition.

01-232

A GENUS OF NEOTROPICAL SEPSIDAE WITH NEARCTIC
AFFINITY (DIPTERA, SCHIZOPHORA)

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A new genus and species of Neotropical Sepsidae (Diptera, Schizophora), were described based on material from Nicaragua (Silva, 1995). The holotype is preserved in the "Museo Entomologico", Leon, Nicaragua. According to the present classification of the family, gave by Steyskal (1987), the new genus has to be included in the subfamily Sepsinae, tribe Sepsini. The genus is characterized by the following apomorphies: abdomen with two constrictions; the hamate surstylus, free from the hypopygium. It runs to Sepsidomorpha Frey in the key of Steyskal (1987) to all genera of Sepsidae known to occur in the Americas. However, it was took the view of Hennig (1949), making Sepsidomorpha synonym of Sepsis Fallén, into account. It is considered here that the features used by Frey (1908) to characterized this genus, namely, the simple male fore femur and the absence of abdominal macrochaetae, are all plesiomorphies. Steyskal (1987) indicated the need of a new genus for the Nearctic species of Sepsidomorpha. The study of the holotypes of the Nearctic species of Sepsidomorpha (S. brunnipes, S. piceipes and S. secunda), described as varieties of S. secunda by Melander & Spuler (1917), confirmed the view that they are congeneric with this new genus. It is the first Neotropical sepsid genus showing a relationship with the Nearctic fauna. The Neotropical region contains six endemic genera but Meroplius Rondani with a worldwide distribution. The new genus described has few representatives in the Neotropical collections in the same way as the Nearctic species, as stated by Steyskal (1987).

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01-231

NEW SPECIES OF METEORIDEA ASHMEAD (HYMENOPTERA:
BRACONIDAE, METEORIDELINAE) FROM NEOTROPICAL REGION.

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The genus Meteoridea is known from the Nearctic (2 species), East Palearctic (1 species), Oriental (1 species), Afrotropical (2 species) and Australian (1 species) regions. The species described here are the first record for the subfamily from Neotropical Region and show that the subfamily extends into temperate regions. They are: Meteoridea whartoni, from Brazil and M. achterbergi, from Brazil and Panama. These species differ from the other species in having discoidal cell sessile, first tergite longer than apical width, 2nd cubital cell narrower above than below, 2nd tergite smooth and the head yellow with the stemmaticum black. M. whartoni and M. achterbergi differ each other by body length and wing venation.

01-233

PHYLOGENETICAL RELATIONSHIPS AMONG CARABUS SPECIES
OF THE SUBGENUS PLATYCARABUS MORAWITZ, 1886
(COLEOPTERA: CARABIDAE: CARABINI).

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Platycarabus Morawitz, 1886, is a highly homogeneous, monophyletic unit among the section "Lobifera" of the genus Carabus Linné, 1758 (in the widest sense), with five species distributed in montane forests and alpine pastures in several massifs of Central and Eastern Europe. The relationships of these species are extremely close, and this fact is demonstrated, besides morphological characters, by the fact that each is able to hybridize with the others in case of syntopy (rare natural hybrids are known from depressus x cychroides, depressus x fabricii, fabricii x creutzeri, creutzeri x irregularis). Nevertheless, the real phylogenetic relationships among Platycarabus species, sometimes hidden by adaptive features in relation to specialized helycophagous diet, have not so far been clarified.

A cladistic analysis of all Platycarabus species has been conducted, based on both adult and larval characters, the polarity of which has been based on outgroup comparisons (Chaetocarabus Thomson, 1875, Hygrocarabus Thomson, 1875, and a Tribax-like, hypothetical outgroup).

Furthermore, a DNA study was carried out using PCR and sequencing the subunit 1 of the mitochondrial NADH gene. The number of variable positions is low due to the very close relationship of the species.

The aim of this paper is to propose realistic hypotheses about zoogeographical and adaptive processes in Platycarabus based on a tree inferred by morphological and molecular data.

01-234

CLADISTIC ANALYSIS OF THE CARABID BEETLE GENUS *AGONUM* BONELLI FOR THE WORLD (COLEOPTERA: CARABIDAE: PLATYNINI)

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The carabid beetle genus *Agonum* Bonelli possesses a collective Holarctic distribution, with limited species diversity also in the rift mountains of eastern Africa and montane regions north of India and southeast Asia. The genus is characterized, among contribal Platynini, by synapomorphies including an unconstricted neck, and a female spermatheca with duct longer than the apical reservoir. Cladistic analysis utilizing the parsimony criterion was conducted for 129 terminals based on 115 potentially synapomorphous morphological characters. Based on the taxon-area cladogram, the genus appears to have radiated after the Eocene opening of the Atlantic Ocean, as all Holarctic relationships are consistent with Beringean vicariance. The major division in the genus sets the European subgenus *Agonum* plus the Holarctic subgenus *Europhilus* as the sister group to clades distributed in Africa, Asia, Europe, and North America. In general, smaller clades are restricted to limited geographic ranges, however strikingly disjunct sister species also occur: e.g., *A. belleri* Hatch from northwestern North America versus *A. ericeti* Panzer from Europe, and *A. gracilipes* Duftschmid from western Palearctic versus *A. placidum* Say from North America. Relationships demonstrated by the cladogram confirm recent synonymical decisions made by J. Schmidt (Germany). A working subgeneric classification is proposed that is contingent upon examination of the as yet unstudied species.

01-235

A PHYLOGENETIC HYPOTHESIS FOR SELECTED GENERA OF PTEROSTICHINE GROUND BEETLES: IMPLICATIONS FOR LARVAL AND ADULT CHARACTER SYSTEMS

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Adult external, male and female genitalic, and larval characters were analyzed cladistically using parsimony methodology for exemplars of nine genera of Pterostichini (Coleoptera: Carabidae), including examples of 15 subgenera of *Pterostichus*. The primarily South American genus *Abaris* was also included in this analysis, the only currently recognized genus of Pterostichini in South America for which the larval form has been described. For the three character systems the relative levels of homoplasy and grouping information was compared. Larval characters are useful for resolving generic level relationships, however, larvae remain undiscovered for most pterostichine genera. Analyses that included genera for which larva are unknown resulted in a loss of resolution. Results from this preliminary study, based on genera for which larval descriptions exist, suggest the polarity and level of stability for various adult character systems in pterostichines. This information was then used to interpret characters in problematic taxa such as *Abropus*, *Metius* and several Australian, African and Asian genera. Results of this study suggest Pterostichini, as presently conceived, is a paraphyletic assemblage relative to other tribes considered to be closely related. A broad and rigorous analysis, that includes multiple character systems, is needed to establish monophyly for a revised concept of Pterostichini relative to putative tribes Loxandriini, and Abacetini, Metiini(=Antarctiini).

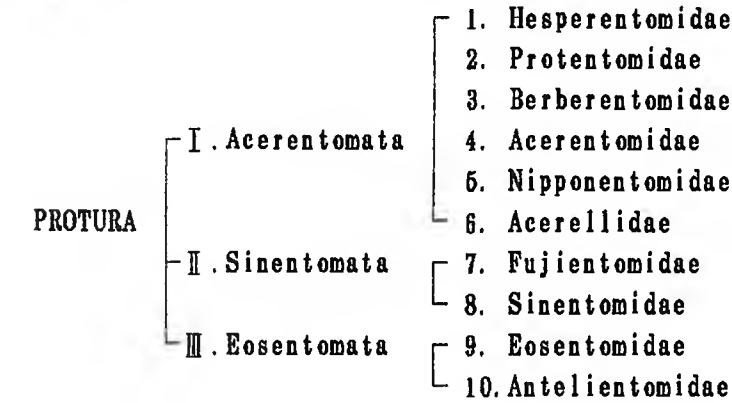
01-236

NEW CONSIDERATIONS ON SYSTEMATICS OF PROTURA

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Recently, the new findings on comparative morphology and spermatology of Protura were shown that the spermatozoa, the pseudoculi, the female external genital organs and the structure of the alimentary canal are all in three major types. These evidences let us reconsidering the conventional taxonomic system of Protura, and a new system was suggested as the following:



01-237

The Systematics of the Scarabaeoidea (Coleoptera)

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A study was made of the articulation and base of the hindwings of Scarabaeoidea (Polyphaga: Coleoptera). Relationships among all families and all Scarabaeidae subfamilies are examined for the first time. The reconstructed phylogeny shows that the Scarabaeoidea is comprised of three major lineages: a glaresid, passalid and scarabaeid lineage. The glaresid lineage consists of only the Glaresidae. The passalid lineage is comprised of two major lines: a passalid line (containing Passalidae, Lucanidae, Diphylostomatidae, Glaphyridae, Trogidae, Bolboceratidae and Pleocomidae) and a geotrupid line (containing Geotrupidae, Ochodaeidae, Ceratocanthidae and Hybosoridae). The scarabaeid lineage contains those taxa traditionally included within the Scarabaeidae (Aegialiinae, Aulonocneminae, Aphodiinae, Scarabaeinae, Orphninae, Melolonthinae, *Acoma*, Chasmatopterinae, Hopliinae, Onccrinae, Rutelinae, Dynastinae, Trichiinae, Cetoniinae and Valginae). The importance of wing characters in constructing higher-level phylogenies, instead of employing the "total evidence" approach, is highlighted.

01-238

PHYLOGENY AND EVOLUTION OF THE TRIBE SCAPHISOMATINI (COLEOPTERA: STAPHYLINIDAE: SCAPHIDIINAE)

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Leschen and Löbl (1995) examined the phylogenetic relationships of the fungus feeding staphylinid subfamily Scaphidiinae (consisting of the tribes Cypariini, Scaphiini, Scaphidiini, and Scaphisomatini). In their study, however, Scaphisomatini was treated superficially and was represented in the data matrix by two terminal taxa. We conducted a preliminary cladistic study of Scaphisomatini (which includes about 35 described genera) by including two genera from each of the subtribes (Heteroscaphina, Baecoceridiina, Baecocerina, Scaphisomatina, and Toxidiina). This analysis, based on 11 terminal taxa (including a generalized outgroup) and 34 characters, resulted in three parsimonious reconstructions of relationships. Each of these show that all subtribes are polyphyletic with the exception of Baecoceridiina which in two trees are monophyletic and sister taxon to *Scaphisoma* (Scaphisomatina). These data suggest the current classification is unsatisfactory and must be changed to reflect natural groupings. Our results are interesting because most members of Baecoceridiina are associated with Old World termites suggesting that the evolution of social insectinquilism may have evolved one time in the subfamily.

01-240

A CATALOGUE OF ORIBATID MITES (ACARIDA, ORIBATIDA) OF THE ARGENTINA REPUBLIC

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The knowledge of the oribatologic fauna of the Argentina republic starts in the beginnings of the last century, in a disorganized way with general studies. In this way are described more or less 40 species essentially from La Plata, Tierra del Fuego, Antartida and Islas subantarticas. Unfortunately the material is not conserved in a good way or the description are incomplete making unused a great majority of them. A second stage in this study come with Marie Hammer investigations along the Cordillera de los Andes, who collected and described material in Salta, Mendoza and Patagonia (Hammer, 1958, 1962). By the same time (Balogh and Csizsar, 1963) described material collected by Topal in El Bolson (Rio Negro). Between 1966 and 1967 Balogh lead the first expedition Hungara of Zoology of southamericans soils, collecting in our country, in Tanti, Sierras de Cordoba (Balogh and Mahunka, 1968). This second stage increased the knowledge of the oribatid mites in our country, counting more or less 140 species, lots of them knew for the science. In the following decades start the work of local acarologists, still in process, with description of species of Mendoza (Fernandez 1987), and the provincia of Buenos Aires (Baranek, Alzuet, Fernandez) principally. The objective of the present work is to present a list of the species of oribatid mites founded up to the present in our country, knew or not, to be used as a starting point, for future investigations. For this, we took as base the work of Balogh and Balogh of 1988, adding those species that for having being published in magazines of local circulation had not been considered, as those had being described with posteriority to its publication.

01-239

UNCOVERING THE RADIATION OF NEW ZEALAND'S ONYCHOPHORA USING MOLECULAR DATA.

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The Onychophora are an ancient Gondwanic group which have been described as the missing link between arthropods and annelids. In New Zealand, five species of Onychophora, belonging to two genera have been formally described. Four species within the *Peripatoides* genus are endemic, with the oviparous *Ooperipatellus insignis* also found in Victoria, Australia. The genus *Peripatoides* is distinctive from all Australian genera but shares features with two other genera, one from Chile, and the other from New Guinea. Morphological taxonomy has proven difficult with this group as few non-conflicting characters exist. However molecular data in conjunction with further morphological information has indicated that New Zealand supports more species than are currently recognised. This talk presents the phylogenetic relationships within the New Zealand Onychophora based on current molecular data. The biogeographic implications of these relationships will also be discussed.

01-241

PHYLOGENY OF THE GENERA OF THE ALEOCHARINE TRIBE MYLLAENINI AND RELATED GROUPS (COLEOPTERA: STAPHYLINIDAE: ALEOCHARINAE)

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Phylogenetic analysis based on adult morphological characteristics provides strong evidence that the genera of the tribe Myllaenini (5 genera, including 2 previously undescribed ones) as well as several genera currently placed in the Diglottini and Pronomaeini form a monophyletic lineage. This lineage is confirmed to be a very early branch of the "higher aleocharines" with which its members share the presence of a tergal gland that opens on the anterior margin of abdominal tergum VII. The Myllaenini are not closely related to the Gymnusini and Deinopsini as proposed by some; however, the phylogeny provides considerable insight into character evolution among basal lineages of aleocharines.

01-242

A REMARKABLE BRACHYPTEROUS FEMALE OF DILARIDAE
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In July 1992 one brachypterous female of Dilaridae was found in a pitfall trap placed in holm-oak wood on Lepini Mountains (Latium, central Italy)*. In the same trap, 4 males were collected too. They belong to the taxon *Dilar parthenopaeus* Costa, 1855. Up to now the female of this species was unknown.

This female specimen (about 6 mm long, ovipositor included), is utmostly more massive than male: head, torax and abdomen were shorter and stouter. The three tubercles on vertex are much more developed whereas eyes are smaller than male one's. The flagellum of antennae is 20-segmented. Mouthparts are well developed. Fore wing (about 4 mm long) is clearly convex, unfit for flight, with thickened membrane and simplified venulation, fainted in medial space. Only one black horny spot (nygma) between MA and MP. Pigmentation stronger and sharper than male one's. Hind wing (about 0,5 mm long) is reduced to a small sub-triangular membranous scale with some veins just outlined. Legs are just a little shorter than male one's.

Life history of this species remains completely unknown.

Presence of brachypterous females with short mobility could have remarkable consequences on population biology of Dilaridae with geographic isolate of uncertain taxonomic range. From there, the hypothesis that *Dilar parthenopaeus* s.l. could be a group of sibling species (or subspecies), as recently advanced (U. & H. Aspöck, 1995 - *Dilar duelli* n. sp. - Z. ArbGem.Österr.Entomol.; Principi & Pantaleoni, 1995 - Checklist delle specie della fauna italiana. 62), is further strengthened.

* In the frame of Researches on Forest Entomocoenosis of central Italy, Dip. of Animal and Human Biology, Univ. Roma, Prof. A.Vigna Taglianti & coll.

01-244

THE PHYLOGENETIC TREE OF HOLARCTIC GENERA OF DIAMESINAE AND PRODIAMESINAE

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The phylogenetic tree of the holarctic genera belonging to the subfamilies of Diamesinae and Prodiamesinae (Diptera, Chironomidae) using all 3 metamorphic stages (larvae, pupae and adult males) is revised. The phylogenetic relationships between genera are still uncertain. Homologies of the hypopygial appendages (aedeagal lobes, volsellae) between the 2 subfamilies are not yet established. It is not yet clear whether basal plate and median field sensu Hansen & Cook (1976) described within the Diamesinae are homologous or not to superior and inferior volsella described within the Prodiamesinae. Phylogenetic reconstruction's are based on male adult morphology, pupal morphology is sometimes included to support conclusions based on adult males. Larval morphology is often not considered in phylogenetic studies, even if the larvae of many genera are still described. The importance of female genitalia is a questioned point. At present phylogenetic relationships based on all semaphoronts are recommended. Some questions are here discussed: the relationships between the genera belonging to the 2 subfamilies and the genera *Pseudodiamesa*, *Diamesa* and *Lappodiamesa*. According to Serra Tosio (1967) *Lappodiamesa* and *Diamesa* are more closely related to each other than with *Pseudodiamesa*, the same point is not shared by Saether & Willassen (1988) that describing the larva of *Lappodiamesa* emphasise the affinity between *Lappodiamesa* and *Pseudodiamesa*. In the present study 120 characters from 19 holarctic genera belonging to larvae, pupal exuviae and adult males are considered. As a preliminary approach a numerical taxonomy study has been carried out to reveal the relationships between genera.

01-243

A COMMUNITY PARASITOID WEB: GALLMIDGES (DIPTERA: CECIDOMYIIDAE) ON *SALIX* SPP. (SALICACEAE). I. STRUCTURE OF THE PARASITOID COMMUNITY

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Within the scope of the research-project „Structure and dynamics of phytophagous systems on *Salix* spp.“ the parasitoid community of eight gallmidge species on willow were analyzed. 7 host species belong to the genus *Dasineura*, formerly *Rabdophaga*, and 1 host to the genus *Iteomyia*. The plant galls were collected on about 25 different localities in Germany, Denmark, Swiss and Italy in the season of 1994/95. During that period 25 Chalcidoidea and at least 5 Platygastroidea were reared.

Most of the chalcid parasitoids seems to be idiobiont species (do not permit the host to grow after parasitization) but more examinations could raise the real part of Koinobionts (allow their hosts to continue to feed and develop after parasitization) in the community. 92 % of the chalcid species parasitized just one of the investigated hosts. The genus *Aprostocetus* dominate the community and is represented by 10 species.

The Platygastroidea are Koinobionts. All *Dasineura* spp. are parasitized by them. Their taxonomic situation is difficult but all of them belong to the 3 genera *Platygaster*, *Inostemma* and *Synopeas*.

Further investigations will examine the structure and organization of the different guilds (e.g. leaf-galls versus stem-galls) over at least three years and the response of the parasitoid community to host population dynamics. The final goal is the construction of a quantitative parasitoid web dealing with gall-inducers on *Salix* spp.

01-245

THE PRELARVAE OF ORIBATIDS MITES
(ACARIDA: ORIBATIDA)

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The knowledge of the existence of prelarvae in Oribatid mites, date back to 1855, when Nicolet describe them for the first time. Claparede in 1868, and Michel in 1884 and 1888, who drew other prelarvae (under the name of deutovum).

A series of studies commenced in 1938 with the work of Grandjean. The definition of the calyptostase was created (Grandjean, 1938, 1954, 1958, 1962); Van der Hammen, 1963, Lions, 1967, 1968, 1971, 1973). In spite of their importance as far as evolutive and phylogenetic studies are concerned, our knowledge at present is still fragmentary, only works in Argentina the Fernandez et al, 1991 and Velis in press.

Morphological survey was carried out the inferior and superior oribatids mites prelarvae. It was discussed that discordant evolution is consistent with life-span as adaptative strategy. Discussion was also made on character evaluation of prelarvae.

01-246

REVISION AND TRANSFERENCE TO RHOPALOPHORINI (COLEOPTERA, CERAMBYCIDAE, CERAMBYCINAE) OF THE GENERA *COREMIA* A.-SERVILLE, 1834 AND *MEROCOREMIA* MARQUES, 1994.

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A detailed morphological comparative study of the species up to now included in the genus *Coremia* together with *Merocoremia* and representatives of Rhopalophorini and Compsocerini showed: 1) significant differences among the present recognized *Coremia* subgenera; 2) the closer affinity of *Merocoremia* and of the subgenera of *Coremia* to the Rhopalophorini genera *Rhopalophora* and *Cosmisoma*, the similarities with *Compsocerus* and allied Compsocerini genera being symplesiomorphies. Thus the present subgenera of *Coremia* are being ranked to the genus status and transferred to Rhopalophorini together with *Merocoremia*. The genera *Coremia* A.-Serville, 1834, *Dirocoremia* Marques, 1994, stat. n., *Thalusia* Thomson, 1864, stat. n., *Lathusia* Zajciw, 1959, stat. n. and *Merocoremia* Marques, 1994 are redescribed including characters of the mouth pieces, endosternites, wing venation, empodium and male and female terminalia. In spite of the differences observed among these genera, a set of apomorphic character states is shared by the here called *Coremia* group and may probably indicate its monophyletic condition within Rhopalophorini. A key to the genera and species is added.

01-248

RECONSIDERATION OF THE SYSTEMATIC POSITION OF FOUR SPECIES OF *CYRTONEURINA* GIGLIO-TOS, 1893 (DIPTERA, MUSCIDAE)

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Among the forty-seven species listed in Carvalho et alii 1993, a catalogue of the Muscidae of the neotropical region, the following four species mentioned have a new status. Holotypes, syntypes and specimens compared with the types were examined as part of a study of the whole genus and every morphological character used was taken into account. The new combination *Cyrtoneurina bequaerti* (Aldrich), 1932 is not a *Cyrtoneurina* as it has striking differences, and for instance can be cited: anepimeron well setulose; setae below the lateral bristles of the scutellum absent; dm-cu strongly curved; arista with short hairs; body length more than 7,0 mm. *Cyrtoneurina stellata* Couri, 1892 is actually *Polietina* Schnabl & Dziedzicki, 1911 as it has also remarkable differences with *Cyrtoneurina* for example: gonopod is too big in proportion to the dimension of gonopod; distiphallus is too big; cercal plate has spines, typical of *Polietina*; 3 notopleural bristles; calyptra setulose. *Cyrtoneurina geminata* (Stein), 1904 is not a synonym of *Cyrtoneurina gemina* (wiedemann), 1830 as it has a prosternum bare; R₁ dorsal and ventral surface bare; dm-cu straight; about 14 hairs on arista; midtibia at anteroventral surface without bristle on medium third.

01-247

BIOCHEMICAL SYSTEMATICS OF TINEIDAE (LEPIDOPTERA)

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Although analysis of DNA has had a profound impact on biosystematics, the potential of other biochemical systems has not been thoroughly investigated. Yet, insect cuticles contain complex mixtures of organic molecules that are potentially useful taxonomic characters. The few studies that there have been of these low-volatility, long-chain organic molecules have highlighted their potential contribution to taxonomy but have left a very incomplete picture of their distribution in the Lepidoptera.

A group of marker compounds were extracted from Tineidae and analysed using Gas Chromatography-Mass Spectrometry (GC-MS). Area normalised chromatogram peaks were used to derive a quantitative measure of marker compounds in individual specimens and statistical analysis used to analyse patterns of their occurrence at individual, species, generic and higher taxonomic levels. These patterns are discussed and assessed against phylogenetic evidence from morphological characters.

01-249

DENSITY-RELATED INTERACTIONS AMONG GALLING INSECTS

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Many galling insects seem to have a limited capability to disperse. This often leads to high local densities at different scales, for example, on a plant, a branch or a leaf. In this study we have surveyed how density affects performance of galling insects in six different systems: cecidomyiids on aspen, cynipoids on oak, a cecidomyiid and a tenthrinid on Salix and an adelgid on spruce. Gall size was the performance parameter studied in all systems. In some systems we also studied the effect of density on survival of insects initiating the gall. The plant unit studied in most systems was single leaves, but included in some also larger spatial scales. Our data indicated that, in some systems, there could be a positive effect of density on performance up to a certain point, beyond which the relationship became negative. Preliminary results on the relative strength of intra- and interspecific competitive interactions are also presented. Possible mechanisms behind and consequences of these cooperative and competitive interactions among galling insects are discussed.

01-250

THORACIC SCLERITES: ADDITIONAL INFORMATION CONCERNING THE BASAL EVOLUTION OF DIPTERA (INSECTA)

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Thoracic sclerites of different basal families of Diptera were studied. They presented useful information concerning the discussion about the phylogenetic relationships among the basal stems of Diptera. The most helpful feature is the meron, which may be attached to the mid coxa (plesiomorphic) or separated from the coxa, fused in various degrees to the thoracic sclerites (apomorphic). The Tipulomorpha—including Tipulidae and Trichoceridae—and the Bibionomorpha—including Pachyneuridae, Cramptonomyiidae, Anisopodidae, Bibionidae and the Mycetophiliformia—are plesiomorphic for this fusion. A meron fused to the thoracic sclerites is clearly seen in the Ptychopteromorpha, Psychodomorpha (without Trichoceridae and Anisopodidae), Culicomorpha, Axymyiomorpha and Brachycera. This partially agrees with previous phylogenies, especially displacing the Scatopsoidea, Perissomatidae and Axymyiidae from the Bibionomorpha to other stems and in the more basal position of the Bibionomorpha in the phylogeny of the Diptera. Apomorphic modifications exclusive of the Bibionomorpha indicate the possible monophyly of the group. A fusion of the epimeron I with the mesothorax may be a homologous achievement of the Scatopsoidea and the Brachycera, indicating that the "Psychodomorpha" may correspond, as recently suggested, to a merophyletic arrangement.

01-252

CLADISTIC ANALYSIS OF SCIOMYZIDAE FALLÉN, 1820 (DIPTERA)

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A previous analysis is presented in order to define the phylogenetic relationships among the genera of the family Sciomyzidae which has a worldwide distribution. The monophyly of Sciomyzidae is based on the habit of malacophagy of the larvae.

The analysis was performed using the program Hennig86. Thirty six morphological and one behavioral character were used to analyse the sister-group relationships among fifty genera.

In the analysis using the successive weighting option, six cladograms were obtained. One consensus cladogram is presented.

The subfamilies Salticellinae and Sciomyzinae and the tribes Sciomyzini and Tetanocerini are monophyletic.

01-251

THE PHYLOGENETIC VALUES OF THE DIFFERENT APPROACHES IN THE HOVERFLIES (DIPTERA: SYRPHIDAE) CLASSIFICATION

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The hoverflies are one of the largest families of the Diptera with more than 5500 already described species. Syrphids are well known for their acrobatic fly and hovering, mimetic coloring and visiting flowers for nectar and pollen. Larvae have extremely diverse feeding habits, with species being saprophages, zoophages and phytophages. This great diversity makes the family very attractive for testing ideas about the evolution of different strategies of development. Many authors deal with problem of hoverflies phylogeny having different approaches in consideration of division and relationship within hoverflies groups. This work concerns the values of different characters used in the hoverflies systematics: adult morphology, especially male genitalia structure, larval morphology, type of larval development, centers of groups origin, etc. We try to emphasize the importance of differences between characters with low and great level of adaptability values.

01-253

THE PHYLOGENETIC POSITION OF THE BAT-FLY FAMILY (DIPTERA: MYSTACINOBIIDAE) FROM NEW ZEALAND.

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A new species of bat-fly, *Mystacinobia zelandica*, was described in 1976 (Holloway, 1976). This fly is flightless with reduced vision and has claws that allows movement over the bat's fur but does not possess mouthparts enabling the piercing of skin. Instead the adults and larvae feed on guano of the New Zealand lesser short-tailed bat *Mystacina tuberculata*. The adults cling to the bat's fur in order to disperse to new roost sites. This species has a level of group sociality not observed in other Diptera including group oviposition, clustering of all stages, mutual grooming, and extension of the male's life-span beyond the reproductive phase to form a sound-producing guard caste.

It is clear that this species does not belong to any blood-feeding Streblidae or Nycteribiidae, nor any other known family of Diptera. Therefore *M. zelandica* exists as the sole representative of its genus and family Mystacinobiidae. Morphological features suggest that this species belongs among the acalypterates with many drosophiloid features hence its current position within the superfamily Drosophiloidea. However, the many novel features which required a new family to be erected have also placed some question as to the phylogenetic position of this family within the Drosophiloidea. Molecular characters have been used to help solve this issue and determine the origin of this distinctive member of New Zealand's unique endemic fauna.

01-254

CLADISTIC ANALYSIS OF SCIOMYZIDAE FALLÉN, 1820 (DIPTERA)

L. Marinoni

Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brasil

A previous analysis is presented in order to define the phylogenetic relationships among the genera of the family Sciomyzidae which has a worldwide distribution. The monophyly of Sciomyzidae is based on the habit of malacophagy of the larvae.

The analysis was performed using the program Hennig86. Thirty six morphological and one behavioral character were used to analyse the sister-group relationships among fifty genera.

In the analysis using the successive weighting option, six cladograms were obtained. One consensus cladogram is presented.

The subfamilies Salticellinae and Sciomyzinae and the tribes Sciomyzini and Tetanocerini are monophyletic.

01-256

BIODIVERSITY AND BIOGEOGRAPHY OF THE UPDATED CHRYSOMELID FAUNA OF BALEARIC ISLANDS (COLEOPTERA)

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The chrysomelid fauna of the six larger islands of the Balearic archipelago is updated. The biodiversity and similarity between islands are quantitatively measured. A particular emphasis is given to the five endemic species so far recorded.

01-255

A NEW BEETLE (*AFROCUPES FIRMAE*) (PERMOCUPEDIDAE) FROM THE UPPER PALAEOZOIC WHITEHILL FORMATION OF SOUTH AFRICAH. Geertsema, J.A. Van den Heever¹

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An extensive array of Permian cupedoid beetles is known from Russia and Czechoslovakia. To date only one cupedid beetle, *Moltenocupes townrowi*, from the Triassic Molteno Formation of South Africa and two permocupedid beetles from the Permian Irati Formation of Brazil have been described. Here a further permocupedid beetle, *Afrocupes firmae*, from the Permian Whitehill Formation of South Africa is reported. The Whitehill Formation is the temporal and lithological equivalent of the Irati Formation and the discovery of *A. firmae* provides further proof of the Parana-Karoo Basin linkage. *A. firmae* is the oldest beetle yet found on the African continent. The paucity of fossil insects from the Whitehill Formation is briefly discussed.

01-257

NEW SPECIES OF *CALYCOMYZA*, *JAPANAGROMYZA* AND *GALIOMYZA* (DIPTERA: AGROMYZIDAE) FROM BRAZIL.

Esposito, Maria Cristina. Departamento de Zoologia, Museu Paraense Emílio Goeldi, Belém, PA, Brazil.

Five new species are described from Brazil: *Calycomyza unicampensis* and *Calycomyza genebrensis*, mining leaves of *Ipomoea batatas* (R.) Poir. (Convolvulaceae); *Calycomyza ipomoensis*, mining leaves of *Ipomoea asarifolia* (Desr.) Roemer & Schultes (Convolvulaceae); *Japanagromyza macroptilivora* mining leaves of *Macroptilium latyroides* (L.) Urban. (Fabaceae) and *Galiomyza richardii* mining leaves of *Richardia brasiliensis* Gomez (Rubiaceae). The *Galiomyza* genus is recorded for the first time in Brazil.

The study indicates the importance of a number of characteristics which, alone or in combination, facilitate the identification of agromyzid species. The most important of these are the morphological characters of the male genitalia, larval behaviour (form and location of mines, appearance of the frass and pupation sites) and the plant species attacked.

01-258

A NEW SPECIES OF *RHYACOPHILA* Pictet, 1834 FROM SOUTHERN ITALY (INSECTA TRICHOPTERA: RHYACOPHILIDAE)

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Istituto di Zoologia, Facoltà di Scienze MM.FF.NN. Università di Perugia. Italy

Rhyacophila vallei n. sp.

Holotype ♂, allotype ♀: Calabria, Gole del Raganello, Civita, 400 m a.s.l., 29.VII.1993, coll. Pantini, Valle. At the Natural Science Museum of Bergamo. Paratypes 2 ♂, 4 ♀ in Moretti's collection at the Zoology Institute, Perugia University.

Male: antennae, palps, legs pale. Wings yellowish with small brown spots. Length of forewing 10 mm.

Genitalia: dorso-apical lobe of segment 9 long, slightly downturned. Upper body of segment 10 dark. Aedeagus curved and apically hooked. Parameres elongated, bifid at the apex with a preapical tooth. Second segment of gonopods markedly concave with strongly protruding inferior lobe covered with thick dense spines on the apical surface.

It is close to *Rhyacophila tarda* Giudicelli from Corsica.

Female: length of forewing 12 mm. Genitalia: at the proximal part of segment 8 a sclerotized collar with a shallow dorsal and ventral split. Spermathecal sclerite elongated club-shaped. Apex of segment 10 bilobed with two short cerci. Last instar larva: length 12 mm. Pale with dark marbling on the abdominal tergites. Head and pronotum with a few small brown spots. Posterior margin of the pronotum large and black. Gills with four filaments each divided by a constriction. Anal prolegs with sabre-like projection, claw with two small teeth.

The species is named after my friend Dr. M. Valle, esteemed Curator of the Natural Science Museum 'Caffi' of Bergamo.

01-260

A NEW CHARACTERIZATION OF *CYRTONEURINA* GIGLIO-TOS, 1893 (DIPTERA: MUSCIDAE)

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In the original description and in the subsequent work, in 1895, Giglio-Tos defined *Cyrtoneurina* with few characters, almost general ones. Other authors that studied this genus, described species and even biological aspects (e.g. Malloch 1925 and Ségué 1937) but it was Snyder 1954 the first author to give a complete definition of *Cyrtoneurina* and to compare it to the related genus. In the present work several characters were used for the diagnosis of the genus and the main ones present in the greater number of the species are: eyes bare or with discrete ciliation among the ommatidia; arista plumose with 9-30 long hairs, with secondary pilosity; Dc 2:4, 2:3; setae below the lateral bristles of the scutellum; prosternum bare, with fine pubescence or setulose; katepimeron bare or setulose; R₁ and R₄₊₅ dorsal and ventral surface setulose; M₁₊₂ not strongly curved towards R₄₊₅; midtibia at posterior surface with two bristles; hindfemur at anteroventral surface with two or three bristles on apical third; hindtibia at anterodorsal surface with one or two bristles; wing spotted or not; angulation on apical half of sternite V present or absent; surstylus bare or setulose, with the tip near or well far from the apex of the cercal plate; eedeagus spines present or absent; ovipositor long or short; epiproct bare or setulose with two bristles; tergites broad or narrow.

01-259

SOME NEW COCCINELLIDS (COL. : COCCINELLIDAE) SPECIES FROM IRAN.

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In 1989-1995 during a faunistic survey for coccinellids in 5 provinces (North, Northeast, Central and southwest) of Iran, altogether 61 species belong to 25 genera from 12 tribes and 6 subfamilies were collected and identified. Among the collected coccinellids, 12 species were new records for Iran as follows:

I- Subfamily: Scymninae

a- Tribe-scymnini

- 1- *Scymnus* (*Scymnus*) *inderihensis* (Mulsant)
- 2- *Scymnus* (*Scymnus*) *levaillanti* (Mulsant)
- 3- *Scymnus* (*Pullus*) *argutus* (Mulsant)
- 4- *Nephus* (*Sidis*) *biguttatus* (Mulsant)
- 5- *Nephus* (*Sidis*) *biflammulatus* (Motschulsky)
- 6- *Nephus* *ulbrichi* (Fursch)
- 7- *Diomus*? *rubidus* (Motschulsky)

b- Tribe-Hyperaspini

- 8- *Hyperaspis* *polita* (Weise)
- 9- *H. concolor* (Suffrian)
- 10- *H. duvergeri* (Fursch)

II- Subfamily: Sticholotidinae

Tribe-serangini

- 11- *serangium montazeri* (Fursch) n. sp.

III- Subfamily: Coccidulinae

Tribe-Novini

- 12- *Nephus fenestratus* (sal.)

The coccinellids prey on aphids, spider mites, coccids, and whiteflies.

01-261

PHYLOGENETIC RELATIONSHIPS WITHIN THE CULICOMORPHA INFERRED FROM 18S AND 5.8S RIBOSOMAL DNA SEQUENCES (DIPTERA: NEMATOCERA)

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We examined the evolutionary origins of mosquitoes by sequencing the entire 18S and 5.8S genes in the nuclear, ribosomal gene array of twelve nematoceran taxa. Maximum parsimony analysis and distance analysis with neighbor-joining demonstrated that a phlebotomine sandfly formed the most basal lineage when a tipulid was used as an out-group. Within the Culicimorpha, corethrellids, chabborids and culicids formed a monophyletic group. Phylogenetic relationships among dixids, chironomids, simuliids and ceratopogonids were unresolved although there was weak support for a sister relationship between the dixid and simuliid. Lack of a spacer in the 5.8S gene was an autapomorphic character among the four genera of Culicidae examined in relation to their sister group, the Chaoboridae. Within the Culicidae, Toxorhynchites was the sister group to an Aedes-Culex clade. The genus, Anopheles formed the most basal lineage among the mosquitoes.

01-262

THE ARMATURE IN THE GENITAL ATRIUM AS A MEANS OF DISTINGUISHING THE PHLEBOTOMINE SAND FLY SIBLING SPECIES *LUTZOMYIA CARMELINOI* RYAN ET AL. AND *L. LENTI* MANGABEIRA (DIPTERA: PSYCHODIDAE) OF BRAZIL

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Recently, the armature of the membrane of the genital atrium has been successfully employed to distinguish females of close related species of phlebotomine sand flies of the Old World genus *Phlebotomus* (Killick-Kendrick et al., *Ann. Trop. Med. Parasitol.*, **88**, 433-437, 1994; Pesson et al., *Ann. Trop. Med. Parasitol.*, **88**, 539-542, 1994). In the present work, the genital area of several Neotropical sand fly species of the genus *Lutzomyia* was investigated to assess the usefulness of this taxonomic character to differentiate morphologically similar or indistinguishable species. Laboratory-reared (n = 20) and wild specimens (n = 10) of a same species from different places in Brazil, including type-localities, were examined by light microscopy. The shape, size and arrangement of spines of each species were compared to those of other species of the same subgenus or species group. To date, distinctive differences in the genital atrium have been recorded only between two species of the *migonei* group, namely *L. lenti* and *L. carmelinoi*. The genital membrane of the former does not appear to have spines apart from a few which can be seen in the edges of the membrane, whereas that of the latter has many long and conspicuous hair-like spines. These features were consistent and could be easily seen in all specimens examined. Therefore, *L. lenti* and *L. carmelinoi*, which have so far been distinguished with certainty only by differences in the length and shape of the genital filament tips of the males, can now be correctly identified even if only females are available. This character may be useful to distinguish other species of difficult identification. Financial support from WHO/TDR (M8/181/4/Q.21) and NIH (AI 16305-17).

01-264

NEWLY RECORDED SPECIES OF HORSE FLIES (DIPTERA: TABANIDAE) IN FAUNA OF CROATIA

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Faunistic collecting of horse flies and their research in the area of Croatia was carried out in the period from 1992 - 1995. The series of new species: *Haematopota bigoti* (GOBERT, 1881), *Hybomitra ucrainica* (OLSUFJEV, 1952), *Aylotus flavoguttatus* (SZILADY, 1915), *Hybomitra expollicata* (PANDELLE, 1883) and *Tabanus regularis* (JAENICKE, 1866) was recorded for Croatia.

Haematopota bigoti the first time was recorded on location in Petrijevci (CR 05) on the following dates: 02.06.1993 (2♀), 19.06.1993 (2♀), 22.06.1993 (4♀), 08.07.1993 (2♀), 09.07.1993 (9♀), 10.07.1993 (7♀), 29.07.1993 (1♀), 12.08.1993 (3♀), 13.08.1993 (1♀), 09.09.1993 (5♀), 25.09.1993 (2♀), 16.07.1994 (4♀), 23.07.1994 (2♀), 24.09.1994 (5♀), 26.05.1995 (2♀), 11.06.1995 (2♀), and Osijek (CR 24), 21.09.1993 (1♀), Staro Štefanje (XL 37), 08.06.1994 (2♀), Borovik (BR 83) 02.09.1994 (3♀), Borovik (BR 83), 12.09.1994 (3♀), Donja Glavina (XJ 71) 21.06.1995 (5♀), Prološko Blato (XJ 71), 22.06.1995 (5♀), Plesmo (XL 41), 30.06.1995 (1♀), Opuzen (YH 06), 26.07.1995 (1♀). All together there were collected 74 specimens.

Hybomitra ucrainica were caught on location in Petrijevci (CR 05) on the following dates: 27.05.1993 (2♀), 29.05.1993 (4♀), 31.05.1993 (1♀), 02.06.1993 (3♀), 10.07.1993 (1♀), 18.06.1994 (2♀), 02.07.1995 (1♀), and Opuzen (YH 06), 30.05.1995

(2♀). All together there were collected 16 specimens.

Specimens of species *Aylotus flavoguttatus* were collected on the location in Čokadinci (CR 04), 30.07.1994 (1♀), Donja Glavina (XJ 71), 21.06.1995 (1♀), Plesmo (XL 41), 30.06.1995 (1♀), Bokšić Lug (BR 75), 02.07.1994 (2♀). All together there were collected 5 specimens.

Hybomitra expollicata species was collected on the location in Opuzen (YH 06) and Modro Oko (YH 06) on May 30, 1995. Only two specimens were collected.

On a pasture beside the river of Cetina in Omiš (XJ 31) on July 26, 1995 two females of *Tabanus regularis* were collected. All in all there were caught 99 specimens, all of them female.

01-263

WEEVILS (COLEOPTERA: CURCULIONIDAE) FAUNA IN KRAGUJEVAC BASIN (SERBIA)

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Kragujevac basin takes the central position in Serbia. The influences from all cardinal points are present, because there are not extremely high mountains around. This part of Europe has had interesting geological history. All this, including intensive anthropogenous activities in last century, results with interesting flora and fauna.

During investigations from 1987 to 1995, on 25 localities, the 355 species, from 80 genera and 22 subfamilies (including Rhynchitinae, Attelabinae and Apioninae) were collected and determined by author.

The basic conclusion is that human activities are not dramatic changed weevil's fauna in Kragujevac vicinity, but caused new quantitative relationships between and in weevils-settlements in different habitats. The very plastic weevil's fauna, with high adaptability, lives here. Will be interesting and necessary to follow it and in the future.

01-265

CONTRIBUTIONS TO TAXONOMY AND BIOLOGY OF MALAYSIAN SOLDIER FLIES (DIPTERA: STRATIOMYIDAE)

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About 300 species of Stratiomyidae have been recorded from the Oriental Region. But there are many more species to be described and there is a considerable gap in our knowledge concerning the biology of the Oriental Stratiomyidae, the larval stages and the relationships between the genera.

During an ongoing study at the Ulu Gombak Field Studies Centre, near Kuala Lumpur, the senior author reared 11 species of soldier flies from phytosaprophagous larvae associated with decaying bamboo. Seven additional species were collected from other habitats in Gombak, e. g. deserted bee nests, or during field trips to the Temengor Forest Reserve (West Malaysia) and Poring Hot Springs (Sabah, East Malaysia).

We studied the biology of the soldier flies in some detail (e.g., habitat preference, territoriality) and described five new species: *Odontomyia latitibia* (Stratiomyinae), *Ptecticus brunetti*, *P. flavifemoratus*, *P. malayensis* and *P. proximus* (Sarginae). Descriptions of larvae and new biological data were published for *Camptopteromyia fractipennis* (Pachygasterinae), *Ptecticus longipennis* and *P. malayensis*. Further adult and larval descriptions are being prepared for 5 species of *Ptecticus* and several species from the subfamilies Hermetiinae and Pachygasterinae. The collected material enabled us to prepare identification keys for *Ptecticus* and other genera, helped to delimit more precisely some genera in both the larval and adult stage and markedly extended the present knowledge of the Malaysian Stratiomyidae fauna.

01-266

A TAXONOMIC SYNOPSIS OF THE BETHYLIDAE (HYMENOPTERA: CHRYSIDOIDEA) OF THE ORIENTAL REGION AND THE EASTERN PART OF THE PALAEARCTIC REGION

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The present status of the classification of the Bethylidae of the Oriental region and the eastern part of the Palaearctic region is briefly reviewed.

Two hundred sixty-four species of 38 genera in 5 subfamilies are listed until 1993: 252 species in the Oriental region and 14 species in the eastern part of the Palaearctic region.

At genus level, 24 genera belonging to the subfamily Epyrinae, 5 genera to the Mesitiinae, 4 to the Pristocerinae, 3 to the Bethylinae, and 1 to the Galodoxinae are recognized. The Oriental region is richest in genus number comparing with other zoogeographical regions.

Kurian (1954) listed 152 species of the Asian Bethylidae. Almost forty years after, by the publication of the check list of Kurian, only 112 species had been added in this fauna. It is safely said that the Asian bethylid fauna has been left unstudied. Current taxonomic works try to revise Japanese Bethylidae. As a result, 72 species of 16 genera are recognized of which 63 species (88%) and 8 genera (50%) are newly recorded. The number of species of bethylid wasps in Asia are estimated more than 2,000, belonging to more than 40 genera of 5 subfamilies.

01-268

SYRPHIDS (DIPTERA, SYRPHIDAE) IN UZBEKISTAN

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In Uzbekistan, 130 syrphid species comprising 3 subfamilies, 12 tribes and 35 genera have been recorded. Fifty-five species have been first recorded for Uzbekistan. *Heringia senilis* Sack, *Mallota cimbiciformis* L. and *Xylota segnis* L. are first recorded for Central Asia. The most diverse is the fauna of the middle mountains (117 species or 81%). Of this number, 70 species are encountered only in this zone. In the foothills revealed are 55 species (43%); in tugai and deserts 30 species (25%) and 12 species (9%), respectively. In the cultivated landscape 50 species (38%) are recorded. Six biological groups have been distinguished, which are represented mostly by aphidophages (36%) and phytophages (25,4%), and three ecological groups, in which mesophylls constitute 86% of the total fauna. There are twelve zoogeographic groups. Eastern Mediterranean individuals prevail among the Palaearctic immigrants of the syrphids. This is especially notable for the tribe Syrphini. The species endemism constitutes 30,7%. Most endemics are in the genera *Eumeria*, *Merodon*, *Ceriana* and *Chrysotoxum*. Further studies of the mountain regions may increase the number of the syrphids up to 180-200 species.

01-267

ENTOMOFAUNA OF QATAR : ORHOPTERAN INSECT SPECIES

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The peninsula of Qatar lies between 50°45'E to 51°40'E longitude and 24°30'N to 26°10'N Latitude, it occupies the Midway between shatt El-Arab and strait of Hormmuz. Systematics sampling of insects was commenced by the author from various biotopes of state of Qatar covering wadis, gardens, farms and plains. The collected insects comprises 23 species in 21 genera belonging to 4 families. Belonging these species: *Anacridium aegyptium* L. *Acrida pellucida* Klug. *Schistocerca gregaria* forsska°L., *Sphingonotus savignyi* Saussur, *Truxalis nasuta* L. *Liogryllus biomaculatus* de Geer, *Gryllotalpa africana*, *Conocephalus conocephalus* Linnaeus and other species.

01-269

THE PROTAPION SCHILSKY SPECIES (APIONIDAE, COLEOPTERA) FROM THE ROMANIAN FAUNA.

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Findings concerning the distribution of the Protapion Schilsky species in Romania are presented in this paper. We have mentioned the collecting place and date and also the host plants.

In Romanian fauna the Protapion Schilsky species are relatively well represented. On the basis of carried out investigations it presents the common, scarce and new species from Romania.

For the achievement of this study there have been examined the most important entomological collections from Romanian museums, there have been reviewed the references concerning the distribution of these species on the Romanian territory and there also have been used the author's investigations carried out between 1963-1995.

01-270

THE GRASSHOPPERS AND LOCUSTS OF EASTERN AND NORTH-EASTERN AFRICA

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The acridid fauna of the region (Ethiopia, Eritrea, Somalia, Uganda, Kenya, Tanzania & Zanzibar) includes an estimated 745 species, of which 95 have been described as new since 1982 or are currently in press. At generic level, 28 taxa have been described as new during the same period out of a total of 246. Many more crypto-species and subspecies may await diagnosis, likely recognition being based on presence or absence of adult diapause and genetic compatability linked to seasonal occurrence. Centres of greatest acridid biodiversity can now be recognised within 17 biogeographical subregions. Those exhibiting volcanic activity in geologically recent times have low levels of species diversity and endemism. Ancient orogenic belts, such as those around the periphery of the Masai steppe, along coastal Kenya and Tanzania and at the foot of the Ruwenzori Mts. are rich in endemics. The subf. Gomphocerinae contains genera which extend from Africa to Saudi Arabia, Iran and India. This blurs the distinction between the African and Indian subregions.

01-272

COMMENTS ON CURRENT RESEARCH ON SHOOT FLY TAXONOMY AND BIOLOGY AND SUGGESTIONS FOR FUTURE RESEARCH (DIPTERA: CYCLORRHAPHA)

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Shoot flies of a diversity of families, especially Muscidae, Anthomyiidae, Chloropidae and Diopsidae, are injurious to cereal crops and pasture. Pests species occur wherever such crops are grown. Some species appear to be restricted to specific host plants, whereas others are polyphagous. Much traditional taxonomy based upon museum specimens remains to be done. This is legitimate, but has its limitations. It is by rearing from host plants that sexes of the same species can be definitely associated and the range of alternative host plants established. The taxonomy of immature stages must be taken into consideration to corroborate or disprove species concepts. Within the Chloropinae ovoviviparity has been found to be the developmental strategy in several genera, though to what advantage to the fly remains unknown. In *Atherigona* spp. (Muscidae) bacteria and other micro-organisms undoubtedly play an important part in rendering plant material edible. To identify such micro-organisms associated with individual pest species is a prerequisite to understanding their nutrition and might well prove useful in their control.

01-271

CUTICULAR HYDROCARBONS AND SPECIATION IN THE GENUS *GRYLLOTALPA* FROM ISRAEL AND ITALY

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The *Gryllotalpa gryllotalpa* L. group (Gryllotalpidae: Orthoptera) from Europe and the Eastern Mediterranean includes at least 12 sibling species. The different species have been characterized by different number of chromosomes; from 12 in Western and Central Europe up to 19 and 23 on the Eastern Mediterranean. Some of the species have been discriminated by the chromosome numbers only [*G. quindecim*, *G. sedecim*, *G. octodecim* (Baccetti & Capra)]. In the present study we found that the hydrocarbon fraction of the cuticle lipids provided reliable characters for species recognition in this group. Isolated populations of mole crickets with 2N=23 chromosomes (males) are known from two separate and distinct localities: along the shore of the Dead Sea in Israel and along the shore of a crater lake in the Pantellaria Island, south of Sicily, Italy. The latter population was described by Baccetti & Capra as *G. cossyrensis*. Morphologically the crickets are very similar but their hydrocarbon pattern shows that they are distinctly separate species. These two highly isolated and local populations present an impressive convergent response to harsh environmental conditions including hypersaline soil and high temperature along the Dead Sea and living under rocks at the bank of hot thermal water in the crater lake of Pantellaria.

Section 2

Biogeography and Biodiversity

02-002

RELATIONSHIPS BETWEEN MOLECULAR, HABITAT, AND BEHAVIORAL DIVERSITY USING APHIDS AS A PARADIGM

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As the ability to sequence and manipulate DNA and related molecules and associated genetic information advances, the distinction between genetic diversity and species diversity becomes obliterated. Parthenogenetic aphids with the potential of annual sexual cycles provide unique models for the study of bio/genetic diversity. Genetic diversity of agricultural insect pests is viewed as a negative; born witness to by the over 500 species which have developed resistance to one or more insecticides.

A recent summary of insects overcoming plant resistant varieties or cultivars listed 9 of 14 as aphid species. Such genetically differentiated insect populations capable of feeding ^{on} genetically differentiated cultivars are often referred to as biotypes. We have accumulated 8 such biotypes of greenbugs in our laboratory. Additional differential crop entries would cause an exponential increase in the possible number of biotypes.

Determining the frequencies of greenbug biotypes in a region is important to the success of plant resistance programs. Using differentiating cultivars is laborious; therefore, we have attempted to use a variety of molecular methods which have proven to be effective in differentiating other insect populations for differentiating aphid biotypes. Our sampling and laboratory breeding results with greenbugs will be compared with results from other aphid programs.

02-001

MAINTENANCE AND GOVERNANCE OF INSECT BIODIVERSITY IN AGROECOSYSTEMS

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A position from which Biodiversity can be understood and defended in principle and in practice is developed. First, although Biodiversity has intrinsic value, this is not sufficient to change human practices. If Biodiversity is to be preserved, it must be understood in terms of conditional values to human interests. Biodiversity is ultimately local, site-specific, and intimately tied to "Place". The concept of Place is an historically vital concept in Western culture which, although marginalized during the Industrial Revolution, is increasingly used in the context of literature, art, popular culture, and science. As human populations increase and we are no longer able to "exploit and move" in agriculture, an emerging understanding of Place appears to be have promise for culturally grounding preservation of Biodiversity. Ultimately, the maintenance and governance of insect Biodiversity is linked to the human condition and perspectives, and the emergent sense of Place may be key to the preservation of species and processes. Given the importance of insects in characterizing the fauna, flora (indirectly), and ecology -- these organisms not only critically define; but even become, "Place". As we come to recognize the essential element of insects in defining the Places which societies and communities value, it will become possible to maintain Biodiversity. Thus, entomologists must engage in research and teaching that provides a foundation for the cultural understanding of insects and their essential role in defining the Places we value.

02-003

INSECT BIODIVERSITY AND THE GEOLOGICAL RECORD: IMPLICATIONS FOR THE MODERN ERA

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Macroscopic taxic diversity on land is dominated by insects and their interacting partners, vascular plants. Understanding the origin, evolution, and maintenance of this diversity at geological time scales must include extensive examination of the fossil record of insects and especially their interactions with plants. Although compilations of fossil insect diversity have been derived by systematic studies, recent exploration of the paleoecology insect herbivores and their plant hosts have provided new and valuable data. Both approaches address three questions: Approximately when did recognizably modern terrestrial insect diversity originate? How has this diversity been maintained? Have increasing levels of plant defense occurred as a response to insect herbivore pressure?

Winged insects are not known until the Early Pennsylvanian, at 325 Ma. At that time 11 orders of insects were already established and by the Late Pennsylvanian (c. 300 Ma) detritivores, carnivores and, notably, herbivores were present in Euramerican coal-swamp communities that were characterized by considerable partitioning of live and dead food resources. There is strong evidence for a modern-aspect ecosystem of principally insects interacting with plants in intricate ways, including host-specific partitioning of particular plant tissues, and multiple strategies for extracting food, including boring, piercing-and-sucking, galling, external feeding, spore consumption, and probably leaf mining. *Psaronius* tree ferns and *Medullosa* seed ferns harbored the most diverse of these insect herbivore communities. Subsequent diversification of Mesozoic seed plants accelerated ecologic and taxic diversification of insects, and angiosperm dominance did not result in any major insect feeding novelty. The family-level fossil record indicates this diversification was propelled by increasing rates of origination coupled with minimal rates of extinction.

The myriad interactions among modern insects and plants originated as ancient associations in Pennsylvanian coal-swamp forests and in modern insect groups finely partitioning Mesozoic seed plant tissues. During this time, herbivory levels indicate that plant defenses escalated to respond to increasing herbivore pressure. Although modern-aspect insect diversity began around 300 Ma, recent anthropogenic disruption far exceeds that caused by abiotic disruptions of the geologic past.

02-004

SYSTEMATICS AND INSECT BIODIVERSITY:
CONSTRAINTS, CONSPECIFIC PROBLEMS AND
CONTINUITY
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ABSTRACT NOT RECEIVED

02-005

ARTHROPOD DIVERSITY AND HUMAN AND DOMESTIC
ANIMAL HEALTH: CHALLENGE AND RIPOSTE
E. Munstermann (New Haven-United States)

ABSTRACT NOT RECEIVED

02-006

LANDSCAPE MOSAICS AND INSECT DIVERSITY
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Landscape fragmentation has been a vast unplanned experiment, with insect populations across the world being forced into new geometrical patterns. Yet these patterns are not the same for all species in all areas. Many species do not even see, or respond to, the landscape as we see it. For others, the landscape is variegated rather than fragmented. And for yet others, it does not matter whether the land is trampled by game or by cattle. Then there are others that thrive in a new, exotic plantscape. Conversely, others are devastated by it. So, is there a general paradigm of how insect diversity responds to agricultural and urban fragmentation? This question will be explored here.

02-007

THE ROLE OF GENE DIVERSITY IN ARTHROPOD
BIODIVERSITY
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ABSTRACT NOT RECEIVED

02-008

BIOGEOGRAPHY, BIOLOGICAL DIVERSITY, AND CONSERVATION OF INSECT FAUNAS AND THEIR HABITATS BY TRADITIONAL PEOPLES IN THE NEOTROPICS

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The distribution patterns of butterfly **endemism** at the species and subspecies levels, **diversity** at genetic, species, and community levels, and **rarity** (concentrations of little-known, scarce, or threatened taxa) were analyzed throughout the Neotropics (over 2000 data-points). Regions of maximum values for these important system qualities do not normally overlap, suggesting that they arise by different mechanisms acting over different time-scales. Ancient vicariance through mountain uplift and water barriers correlates with species endemism, while Pleistocene paleoecology agrees with lower-level differentiation. Unpredictable disturbance produces and maintains high beta-diversity, while complex chance processes in scattered small sites allow erratic survival of primitive and rare species in irreplaceable "paleoenvironments".

These non-coincident distribution patterns make the conservation of all these factors very difficult, especially if only in a predetermined system of official conservation units. In such limited areas, the maintenance of natural system processes such as microevolution, colonization, predator and parasite cycles, diversification, and high beta-diversity is problematic. Their management requires the input of much energy, usually resulting in the irreversible simplification and destructuring of the system and of insect communities.

Studies of low-density "rustic" human communities resident in the Neotropics show that they often maintain or even enhance system diversification processes on their traditional lands, through mild disturbance associated with empirically sustainable natural resource use patterns, attained over long periods of selection on their cultural practices and interactions. In contrast, higher-density, market-directed or exogenous societies rarely demonstrate sustainable use of their natural resources, and often destructure the systems to the point where reduction in diversity becomes irreversible. Thus, true conservation can often be attained by protection of such traditional societies and their resource-use patterns, across the landscape and at all scales of time and space.

02-010

ON THE BIOGEOGRAPHICAL ANDEAN SUBREGION

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The Andean subregion is defined within a biogeographical system of the world, where three biogeographical subregions are recognized (Holarctic, Holotropical, and Austral). South America is divided in two subregions: Andean and Neotropical; the former belongs to the Austral region, whereas the latter belongs to the Neotropical region.

Based on distributional patterns of more than 200 genera, species groups, and species of terrestrial and freshwater Arachnida (Araneae, Opiliones, Pseudoscorpionida, Scorpiones, and Solifugae), Crustacea (Decapoda), and Hexapoda (Coleoptera, Diptera, Homoptera, Hymenoptera, Lepidoptera, Odonata, and Orthoptera), the following five provinces are recognized within the Andean subregion:

- (1) Subantarctic: Austral Andes, from 37° south latitude to Cabo de Hornos, including the archipelago of southern Chile and Argentina.
- (2) Central Chilean: Central Chile between 32° and 37° south latitude.
- (3) Puna: Highlands from southern and central Peru, western Bolivia, northwestern Argentina, and northern Chile, basically over 3000 m altitude.
- (4) Patagonian: Semidesert west of the Austral Andes to the Atlantic coast in Argentina, extending in some places to Chile.
- (5) Paramo: Northern Andes from northern Venezuela, Colombia, and Ecuador, over 3000 m altitude.

The Central Chilean and Subantarctic provinces represent the richest areas in endemic species and taxa with phylogenetic value, thus their high value for biodiversity conservation.

02-009

BIOGEOGRAPHY OF THE MEXICAN TRANSITION ZONE: HYBRID AREAS AND GENERAL AREA CLADOGRAMS

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The Mexican Transition Zone contains a biota with diverse historical origins. A diverse endemic biota, existing in the region since the breakup of Pangaea, is complemented by more recent colonization from the north via the Sierra Madre Occidental-Rocky Mountain axis starting in the Oligocene, and from South America via the Panamanian isthmus since the Plio-Pleistocene. Given such a diverse array of biogeographic histories, the search for a general pattern of area relationships as proposed in cladistic biogeography is called into question. Nonetheless, areas of endemism can be defined by mountain system, with many taxa restricted to limited expanses of montane habitat. Using cladistic biogeographic methods, we analyze biogeographic patterns in a diversity of these montane taxa, focusing on Passalidae and Carabidae (Coleoptera), but also including other insects, fish, lizards and plants. Areas of endemism include the Sierra Madre Oriental, S.M. Occidental, southern S.M. Occidental, S.M. del Sur, Sierra Transvolcanica, Chiapas-Guatemala Highlands, Talamancan Cordillera, Arizona Mountains, and Sonoran Desert. We compare estimates of patterns based on members sharing coincident biogeographic tracks to those hypothesized based on data combined from all groups representing all histories. The former analysis may provide better understanding of taxa sharing a similar history, but the latter can serve as a useful tool for identification of areas requiring preservation. By all methods and taking into account the current biosphere network, the region at most risk for massive loss of biodiversity is the Sierra Madre del Sur.

02-011

BIOGEOGRAPHICAL PROCESSES AND SPECIES RICHNESS IN THE NEOTROPICAL INSECT FAUNA

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Faunal specific richness of a given biogeographical unit is currently ascribed to two classes of different causes, i.e. environmental heterogeneity and ecological history. Biogeographical relationships history of the implied area is usually invoked as a causal factor contributing to the taxonomical heterogeneity degree of a given biota and - *a fortiori* - of its entomological component. However, analyzing how homologous groups of Insects are represented in different tropical areas, we are allowed to suppose that these relationships could play a relevant role in determining specific richness too. Such hypothesis is discussed by means of the analysis of some Insect groups showing disjunct distribution in Eastern Asia and Tropical America and of possible effects of climatic belts shifting during past geological epochs.

The main basic concepts advanced are : i) specific distribution area, which is not regarded just as a merely spatial attribute of the species itself, but as the portion of geographic space in which the species is present and interacts in a non occasional way with the ecosystem. Consequently, it has ontological properties; and ii) dynamic vicariance, which is thought as a process implying range splitting rather than barriers' arising in a previously occupied area. In this process entire biota, not single species more or less isolated from their synecological context, are affected.

Dynamic vicariance, acting on a highly anisotropic scenario, is capable of provoking not only qualitative changes in the extant entomofaunas composition, but also the increase of their specific richness.

02-012

COLEOPTERA PASSALIDAE OF THE AMERICAS
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Distribution of Passalidae species in countries of North America, Mexican Transitional Zone, South of Central America, South America and the Antilles was analyzed. Latitudinal and altitudinal limits related to species distribution in the American Continent were determined. Total species number was assessed for each country, and their distributional extent and degree of endemism in relation to vegetation type and microhabitat were identified.

General and particular dispersal patterns at the tribe, genera and species levels are discussed considering group phylogeny and continental geological history. High species richness and high endemism areas were ascertained, as well as those areas needing further exploration.

02-014

BIODIVERSITY AND DISTRIBUTION OF THE NEOTROPICAL GENUS PSEUDOCHEILA GUERIN, 1839 (COLEOPTERA: CICINDELIDAE).
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The genus Pseudocheila was created by Guérin-Méneville for the species Cicindela bipustulata Latreille, 1881, based on material collected by Alexander von Humboldt during his famous expedition to the Nueva Grenada. Seven additional taxa were subsequently described by other authors (Chaudoir, Bates, Dokhtouroff and W.Horn), all of which were later synonymized by W.Horn into bipustulata. Thus the genus Pseudocheila was thought so far to include a single species only (bipustulata) or two species at most (bipustulata and tarsalis Bates, 1869). Surprisingly, a careful revision, based on the study of type and older specimens as well as of large recent material from several sources, made it clear that Pseudocheila, far from being a monotypic genus, comprises no less than 20 different full species (12 of which are being described as new to science), ranging in mountainous areas from Costa Rica south to the Andean slopes of Bolivia. The distribution and the possible origins and mutual relationships of such species are presented and discussed.

02-013

NEW WORLD CARABIDAE (COLEOPTERA) AND FAUNAL CONNECTIONS OF MIDDLE AMERICA
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The native (introduced taxa excluded) carabid fauna of Middle America (including the West Indies) includes the following geographical components: an older one (late Mesozoic- early Tertiary) of indigeneous genera, species groups, and phylogenetically isolated species; and three younger (late Tertiary-Quaternary) elements , regarded as adventive-immigrant, and classified according to source area: South America; North America north of México; and the Old World (Africa, or the warmer parts of the Palaearctic Region). Overall, the immigrant element is predominant, particularly the South American component , as shown by numbers of genera represented in Middle America. Thus, carabid movement in the New World seems to have been principally in a south-north direction, rather than the reverse. This is reflected also in the putatively older, precinctive elements: most of them exhibit southern connections, as well.

02-015

TAXONOMIC AND ECOLOGICAL COMPOSITION OF THE TREEHOPPER (HOMOPTERA: MEMBRACIDAE) COMMUNITIES OF A LOWLAND NEOTROPICAL RAINFOREST
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The treehoppers collected by insecticidal fogging of tree canopies in five neighboring forests at Tambopata, Peru, were examined in terms of richness and abundance of species and guilds, which were defined by the degree of aggregation and ant-mutualism. From sampling above 4725m² of forest floor, Tambopata was estimated to have approximately the same treehopper richness (200) as the entire Nearctic. Species complementarity (distinctness) among the five Tambopata forest types was high, varying from 79% to 95%, but this was partly explained by small sample sizes. The Terre Firme forest had the second smallest sample size but had the most species. Guild composition varied significantly among forest types (p < 0.001). However, the incidence of mutualistic treehopper species did not track the incidence of their mutualistic ant 'partners'. The fogged specimens from Tambopata (TA) were compared to the results from classical collecting techniques in lowland rainforest sites of Brazil (BR, 660 km distant), Ecuador (EC, 1550 km distant) and Colombia (1900 km distant). Species complementarity was relatively low: 0.81 TA-BR (≈ 30% of TA species in BR samples); 0.91 TA-EC (10% of TA species in EC samples). Thus, the pooled Tambopata sample was less distinct from the analyzed Brazil sample, 660 km distant, than were most of the forest types within Tambopata. Although the distant samples were obtained by different methods, supraspecific differences among all of them seemed mostly attributable only to the vertical stratification used in each case. An explicit method is proposed to wed phylogeny and ecology in community diversity studies. Convex guilds are historically tied, ecologically similar groups of species that, consequently, may respond similarly to forces affecting patterns of distribution and abundance. This concept thus has relevance to community diversity studies, which describe and interpret these patterns. Examining the distribution and abundance of treehopper convex guilds facilitated biological interpretation of community guild patterns and suggested that Tambopata treehopper community differences were partly due to phylogenetic effects.

02-016

AN APPROACH TO THE KNOWLEDGE OF THE INSECT FAUNA OF MONTE: A PERSPECTIVE.

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Argentina possesses an extended area occupied by arid lands, which could be grouped in three main arid biomes: Patagonia, Puna, and Monte. Patagonia and Puna are cold steppe, the first of low altitude in the south, and the second in high mountains in the north. The Monte is a warm scrub desert, extended between the Puna and Patagonia, and, floristically, it can be defined as the *Larrea* distribution area, including other Zygophyllaceae as *Bulnesia* and *Plectocarpa*. The Monte is of a particular biogeographical interest because it extends from the neotropical to the antarctic regions. Patagonia and Puna are evolutionarily related, while Monte faunal and floral elements are most closely related to those of Pampa and Chaco. Most of the studies developed in the Monte area deals with vertebrates. Works on Monte insects are increasing, mainly dealing with a particular taxon or a small area, and mostly referred to the northern area of Monte.

The principal objective of this paper is to develop a program in order to increase the knowledge of the Monte insect fauna. It comprises three main points: a) **Biodiversity**: A common idea is that the Monte is a poor Chaco, but nobody has done, particularly for insects, a comparison of species between these two areas. In this paper, a preliminary list of species from Monte is presented and compared with the Chaco area concerning four families of Insects: Formicidae, Chrysomelidae, Curculionidae, and Tenebrionidae. Protected areas of Monte are basically based on landscape, protection of forest or certain species. However, it does not exist a preliminary plan or inventory of insect fauna to create a protected area for insects. b) **Biogeography**: Classically, endemic species or genera defined a natural area. In the Monte no analysis of endemism was ever developed. A preliminary study is developed, plotting on a map the geographical range of species, and then analyzing this information. The analysis of endemism together with phylogenetic studies of several groups will provide the basis to develop the history of the area and its relationships with other areas. c) **Ecology**: Three kind of ecological programs are being developed in the laboratory: a) a soil fauna analysis in different protected areas examining patterns of temporal distribution; b) an exhaustive study of insects associated to *Larrea*; and c) analyses of daily and monthly patterns of activity of leaf-cutting ants. These studies provide information about ecological ranges, insect-plant relationships, species as indicators of environmental changes, and comparison between disturbed and natural areas.

02-017

HABITAT STRUCTURE AND ARTHROPOD DIVERSITY IN ARGENTINE SEMI-ARID CHACO

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In this paper we examine the extent to which changes in habitat structure, arising from logging and grazing, influence the diversity of ground-dwelling arthropods in semi-arid Chaco forest. The structure of four forest habitats is assessed from quantitative measurements of plant species diversity, spatial distribution and architectural complexity. Arthropod diversity is assessed according to the abundance of different families and functional groups (e.g. predators, detritivores, herbivores). The data indicate that the physical structure of the habitat, architectural complexity and height profile, exerts the strongest influence on the arthropod community; family diversity was significantly smaller in the structurally simpler habitats. Such habitats also exhibited a significant change in the functional composition of the arthropod community.

02-018

BEE AND PLANT DIVERSITY IN THE SOUTHERN REGION OF THE ATACAMA DESERT OF CHILE

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In the arid site South of Copiapó (lat 27° 18' S, long. 70° 25' O), during the blooming season of 1989 and 1991 were found:

1) A desert scrub corresponding to only one community with 6 main species, being *Nolana rostrata* (Nolanaceae) notoriously predominant, followed by *Argylia radiata* (Bignoniaceae) and other less abundant scrub and herbaceous species. 2) The associated bees to this vegetational community belong to 27 species and 6 families. 3) The plant species most preferred by bees, because of their highest diversity, was *Encelia oblongifolia* (Compositae) in 1989, and *Argylia radiata* in 1991. 4) During the latter year a higher diversity and abundance of bees were found, probably due to more favorable climatic conditions because of greater precipitation during winter. 5) The main bee species visiting *Argylia* were: *Leiproctus atacama* (27,42%), *Diadasia nemaglosa* (19,35%) and *L. herrerae* (11,29%). 6) *Encelia* was mainly preferred by: *Centris chilensis* (20,37%), *Megachile saulcyi* (18,52%) and *Mesonychium wagencknechti* (16,67%). Specific pollinators should be expected to be important in habitats like deserts, where their food resources, which they depend upon, are only available for short periods. Among the oligolectic bee species, *Diadasia nemaglosa* was found to be highly specialized on *Argylia*.

02-019

BIODIVERSITY OF THE ANTS (HYMENOPTERA: FORMICIDAE) OF THE VENEZUELAN ANDES.

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The Venezuelan Andes have about 250000 km² or near one quarter of the total country land. The altitudinal range vary from 0 m to 5007 m above sea level. There are 15 life zones, from xerophytic areas to nival zones, rain and cloud forests. These conditions favour a high biodiversity both in richness and endemism, especially shown by the ants. There are species from the Caribbean, the Amazonian and the savannahs areas, as well as the typical Andean and Cis-Andean species. The ants in the author collection, other collections and references are catalogued. Up to date there are catalogued around 60 genera, about 120 species determined and more than 120 species to be determined, some genera (*Camponotus*, *Pheidole*, *Solenopsis*, etc.) will give many species. The Ponerinae have 14 genera with 29 species determined and more than 20 to be determined. The Myrmicinae have 28 genera with 70 species determined and more than 40 to be determined. The Pseudomyrmecinae have one genus and more than 15 species to be determined. The Ecitoninae have 5 genera with 19 species determined. The Formicinae have 5 genera with more than 30 species to be determined. The Dolichoderinae have 6 genera with 21 species determined and more than 10 to be determined. Information is given on their geographical distribution in the Neotropical region and in Venezuela. Localities in the Andes are given with the number of samples for each locality. Ecological data such as altitudinal range, habitat, microhabitat, life zones, as well as ethological data such as the occurrence are given. Biogeographical maps are presented.

02-020

BIODIVERSITY OF THE ANTS (HYMENOPTERA: FORMICIDAE) OF TRINIDAD AND TOBAGO. CATALOGUE AND DISTRIBUTION.

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A Catalogue of the ants of Trinidad and Tobago is given, including species in the author's collection as well as other cited in the literature. There are 7 subfamilies with 66 genera and a total of 238 species determined. The Ponerinae have 16 genera with 37 species. The Cerapachyinae have 1 genus with 2 species. The Myrmicinae have 34 genera with 100 species. The Ecitoninae have 3 genera with 14 species. The Pseudomyrmecinae have 1 genus with 18 species. The Formicinae have 5 species with 35 species. The Dolichoderinae have 6 genera with 22 species. The genera with more species are: *Camponotus* (21 species), *Pseudomyrmex* (18 species), *Azteca* (12 species), *Pheidole* (11 species), *Strumigenys* (11 species) and *Trachymyrmex* (10 species). Bibliographic and ecological information on each species is given, such as references to original description, distribution in the Neotropical Region, and in Trinidad and Tobago. For each part of the country, the localities and records number are included. Also ecological data such as altitudinal range, habitat, microhabitat, life zones, as well as other ethological data such as occurrence. Maps of Trinidad and Tobago are given with the localities where the different species have been collected or recorded by other authors. List are given of all the ant species recorded in Trinidad and Tobago. A list of the collection localities is given, including the geographic coordinates and the altitudes of each place.

02-022

DISPERSAL OR VICARIANCE? HISTORICAL BIOGEOGRAPHY OF MARINE WATER STRIDERS (HETEROPTERA, GERROMORPHA) IN THE INDO-PACIFIC

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More than 120 species of marine water striders (Hemiptera, Gerromorpha), representing three families and ten genera, are distributed throughout the Indo-Pacific region. They live in marine habitats such as mangroves, intertidal coral reef flats, and the sea surface near coral and rocky coasts. Five species of sea skaters, *Halobates* (Gerridae), have colonized the surface of the open ocean. Adult marine water striders are wingless but may disperse along coasts, chains of islands, and possibly across wider stretches of open sea. Although some species of coral bugs, *Halovelgia* (Veliidae), and *Halobates* are widespread, most species of marine water striders have rather restricted distributions. Based upon distributional data for marine water striders, a number of areas of endemism can be delimited within the Indo-Pacific region. Applying the methods of cladistic biogeography, the observed patterns of distribution are sought explained using both dispersal and vicariance hypotheses. The historical biogeography of marine water striders does not support the traditional division of the Indo-Pacific into the Ethiopian, Oriental, and Australian regions. The distributional patterns are more compatible with a set of hierarchical relationships between more restricted areas of endemism.

02-021

GEOLOGICAL HISTORY OF SE ASIA DURING THE LAST 50 MILLION YEARS

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This talk will attempt to describe the geological development of SE Asia and surrounding regions during Cenozoic time. This region is currently very active and since even present-day motions of plates and smaller crustal fragments are not fully understood it should not be surprising that making tectonic reconstructions becomes more difficult as the age of the reconstruction becomes greater. However, the present movements of major plates, such as the Pacific and India-Australia, are well known in relation to Eurasia, and these provide limits. Therefore by working back from the present-day we can make reconstructions, and the resulting interpretations offer a means of identifying important regional events and highlighting key problems. An animated plate tectonic reconstruction of SE Asia for the period 0-50 Ma will be presented to illustrate this approach (available free for a PC or Mac from <http://glsun2.gl.rhnc.ac.uk/seasia/welcome.html>). In this model, the major Cenozoic event in the region was collision of the Australian continent with a Philippine Sea plate island arc at ~25 Ma (end Oligocene) which resulted in major changes in the configuration and character of plate boundaries, and caused effects which propagated westwards through the region. This collision initiated major strike-slip faulting in northern New Guinea and led to the progressive arrival of Australian microcontinental fragments in Sulawesi, providing possible pathways for migration of faunas and floras between Asia and Australia, but also creating new barriers to dispersal. This new model will be discussed and compared to other geological models for the region, for example, those invoking terrane accretion. The animation offers a visual tool for exploring the consequences and validity of alternative interpretations and may be of some help to biogeographers. It is important to appreciate that the model shows the movement of plates and plate fragments which do not necessarily correspond to areas of land and sea. The geological record is a marine record; former land areas and periods of emergence are recorded by negative evidence, and even when there is a rock record it is often difficult to date. Nonetheless, an attempt will be made to indicate the likely distribution of land and sea although at present much of the information required to do this is not easily accessible or not available. Determining the palaeogeography of the region is a major task for the future. Completing this task, and testing the geological models, are problems to be solved for which evidence from all fields of biogeography is vital.

02-023

ASSEMBLING NEW GUINEA - 40 MILLION YEARS OF ISLAND ARC ACCRETION AS INDICATED BY THE DISTRIBUTION OF AQUATIC HETEROPTERA

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The island of New Guinea consists of the northern margin of the Australian continental craton that has collided over the past 40 million years with a series of migrating island arcs. Each of these arcs has had a separate tectonic history and carried a correspondingly different biota. Recent faunal surveys and phylogenetic analyses, coupled with evolving tectonic knowledge, have now allowed us to identify elements within the New Guinea aquatic Heteroptera biota that appear to be correlated with particular accreted arc systems. In particular, it appears that many Asian-derived groups arrived via a Papuan Arc in the Eocene, while many other distinctively Melanesian groups evolved on an initially isolated Solomons Arc that appeared in the Oligocene. The understanding of this interrelated tectonic and biotic history has in turn permitted a better comprehension of the complex patterns of faunal fusion and disjunction currently present within the Melanesian region.

02-024

BIOGEOGRAPHIC PATTERNS IN INDO-PACIFIC MIRIDAE (HETEROPTERA)

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The methods of cladistic biogeography are applied to selected monophyletic groups of Miridae (Heteroptera) in south-east Asia and the tropical Pacific. An overall scheme of area inter-relationships is derived and compared with the results of other studies of aquatic and terrestrial invertebrates. Congruent patterns of distribution in these groups and the recognition of discrete areas of endemism lend support to geological hypotheses concerning the composite origin of the Philippines, Sulawesi, New Guinea, and other complex land masses in the Indo-Pacific region.

02-025

BIOGEOGRAPHIC PATTERNS IN ORIENTAL AND AUSTRALASIAN DOLICHOPODIDAE

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ABSTRACT NOT RECEIVED

02-026

BIODIVERSITY AND BIOGEOGRAPHY OF THE TIGER BEETLES (COLEOPTERA: CICINDELIDAE) OF THE PHILIPPINE ISLANDS.

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The tiger beetle fauna of the Philippine Islands is reviewed and a total amount of 134 different species is recognized (17 of which are still in need to be described). The great majority of these species (i.e. 114 species, the 85 per cent of the whole fauna) is strictly endemic to the Archipelago, and moreover 6 non-endemic species occur here with endemic subspecies. Thus the Philippines appear to be the sixth richest country in the world (after Indonesia, India, Brazil, Madagascar and Zaire) as for the number of inhabiting species, the fifth richest one (after New Zealand, Madagascar, New Caledonia and Australia) as for the ratio of endemic species, but definitely the second richest country as for the number of endemics (just after Madagascar). In a way, it has to be expected that further specialized research will significantly increase such figures in the future, when more islands, other than Luzon, will be intensively explored. On the other hand, however, the ecological devastation that has now befallen most of these islands is likely to rapidly wipe out unknown forest faunal treasures.

The possible origin of the Philippine tiger beetles and their relationships to Sulawesi, Maluku, Borneo and the Asian mainland are also discussed in a broader biogeographical context.

02-027

BIOGEOGRAPHY OF THE BUTTERFLIES OF SULAWESI
R. De Jong, R-I Vane-Wright (London-United Kingdom)

ABSTRACT NOT RECEIVED

02-028

BIOGEOGRAPHY OF SULAWESI BASED ON THE INSECT FAUNA OF LOTIC HABITATS, WITH SPECIAL REFERENCE TO DRAGONFLIES (ODONATA)

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The fauna of Sulawesi is characterized by a high percentage of island endemics. The fact that various speciose groups of Borneo do not occur in Sulawesi (e.g. the family Platynemididae of the Odonata) also indicates to a long period of isolation. Groups of closely related species confined to Sulawesi frequently have small distributional ranges, defining areas of endemism. Island endemism and small distributional ranges within Sulawesi provide the tools for two levels of biogeographical studies. First, a reconstruction of the origin of the Sulawesi fauna, and second, a reconstruction of the relationships of the respective areas of endemism within Sulawesi.

Widespread species occurring in Sulawesi are predominantly Oriental. Most island endemics have their presumed sister groups west of Sulawesi, although in some taxa the relationship between the Sulawesi and Philippine fauna is very close. The species of Oriental genera or species groups distributed from the mainland of Southeast Asia to the Papuan region frequently show a sister group relationship between the Sulawesi taxa and those of the area east of Sulawesi.

Long isolation and a turbulent geological history have obviously provided the opportunities for radiation in those groups that reached Sulawesi, e.g. the damselfly families Chlorocyphidae and Platystictidae. Based on phylogenetic relationships and distributions of the (micro)species involved, taxon-area cladograms will be presented. Relationships between areas of endemism based on lotic fauna largely coincide with reconstructions of the island's history based on geological data.

02-030

NOTES ON THE BIODIVERSITY AND BIOGEOGRAPHY OF XANTHOLININI FROM SOUTH-EAST ASIA: AN EXAMPLE OF A GROUP IN EVOLUTION (COLEOPTERA, STAPHYLINIDAE)

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A revision of south-east Asian Xantholinini (Bordon, in preparation) has revealed the exceptional richness of this tribe of Staphylinidae in one of the largest but least studied regions of the world. Until now about 400 species (70% new) belonging to 39 genera (20 new) have been identified; some are pantropical and a few (in contrast to what was believed) are palaearctic (on the western and eastern boundaries); all the others are typical of south-east Asia and/or of the austral region. The genera with the most species are correlated on the one hand to the great biodiversity of the rain forest with many species living under the cortex of trees or in rotting wood and on the other by the Himalayan zone with humicolous, brachypterous, subapterous and microphthalmic species. This vast territory with its multiple environments is characterised by a great biodiversity, as revealed by the abundance of species and scarce number of specimens, by their diversification and specialisation, and by an evolutive convergence less frequent than what is known in other Staphylinidae. The morphological study shows an extraordinary variety in several structures. The sixth visible urite and male genital segment (sometimes with soldered pleura) can be normal, with the known modifications, or extraordinarily metamorphosed; the basal bulb has lateral lobes which can be normally developed, modified, exceptionally complex, atrophied, or absent; the internal sac can be simple, without any evident amature, with tiny sclerotized spines, or with enormous structures which can extend outside the abdomen. Such variety is the consequence not only of the diverse environments but also of an ongoing and complex evolutive process, about which little is known and much has yet to be learned, markedly characterising a tribe which should be provisionally isolated within the Staphylininae but whose taxonomic position should be reviewed in relation to the other tribes to which it is usually linked.

02-029

THE DISTRIBUTION OF APSILOCHOREMA ULMER, 1907 - BIOGEOGRAPHIC EVIDENCE FOR THE MESOZOIC ACCRETION OF A GONDWANIAN MICROCONTINENT TO LAURASIA (TRICHOPTERA: HYDROBIOSIDAE)

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The genus *Apsilochorema* ULM. contains 42 nominal species. They are distributed over a wide range in continental South Asia, on the Sunda Islands, on the Outer Melanesian Arc and in Australia. The family is one of the most ancestral groups of extant Trichoptera. *Apsilochorema* is the only representative of the family occurring in the Northern Hemisphere. The evidence for a mesozoic immigration is based on the:

- fossil record of the family in Asia from cretaceous amber,
- ancestry of the South Asian species,
- sistergroup relationships on generic level,
- ecological competition with the Holarctic Rhyacophilidae.

02-031

THE IMPACT OF FOREST PLANTATION DEVELOPMENT ON MOTH DIVERSITY IN MALAYSIA.

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Tropical forests that are rich in biodiversity and timber resources have been logged indiscriminately in the past. Logging pressure on natural forest can be reduced with the creation of forest plantations. The impact on the geometrid moth diversity resulting from the creation of two different types of plantations, indigenous and fast-growing exotic, were discussed and compared with an advanced regenerating natural forest. Among the four forest plantations that were assessed, the indigenous plantation was found to support a higher geometrid moth diversity as indicated by Williams alpha diversity index. Second highest in geometrid diversity was the fast-growing exotic plantation planted with *Gmelina arborea* Roxb., followed by *Paraserianthes falcataria* (L) I. Nielson and *Acacia mangium* Willd. The implications of the creation of forest plantations were also discussed in the context of biodiversity and conservation.

02-032

GEOGRAPHIC HISTORY OF THE TROGIDAE (COLEOPTERA: SCARABAEOIDEA)

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The small, cosmopolitan family Trogidae consists of three genera, *Trox* F., *Omorgus* Erichson and *Polynoncus* Burmeister and about 300 species. *Trox* is restricted to the Holarctic (subgenus *Trox*) and Afrotropical (subgenus *Phoberus* MacLeay) regions; *Polynoncus* occurs only in South America; *Omorgus*, with three subgenera, occurs in the Oriental and Afrotropical regions (*Afromorgus* Scholtz), southern Nearctic, Neotropical and Australasian regions (*Omorgus s. str.*), and South America (the monobasic *Haroldomorgus* Scholtz). Relationships between and within genera have been cladistically inferred by analysis of adult and larval characters.

Cladistic and biogeographic evidence points to a Laurasian origin for *Trox* and a Gondwanan origin for *Omorgus*. The less derived subgenus *Trox* is restricted to the Holarctic while the more derived *Phoberus* has clearly invaded and evolved in Africa, after the break-up of Gondwanaland. The most derived *Afromorgus* species are restricted to the African-Oriental regions while those of subgenus *Omorgus* are widespread in southern North America, South America and Australia, pointing to a typical Eocene radiation between South America and Australia and a much more recent invasion of North America from South America. *Polynoncus* and *Omorgus* (*Haroldomorgus*) are South American endemics.

02-034

CLIMATO-ECOLOGICAL ZONATION OF GONDWANALAND

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It has become evident that extant organisms often indicate past land connections between parts of the South Continents. Such distribution patterns are termed Gondwana-type. The frequency and level of phylogenetic relations between separated taxa already helped to determine the sequence of continental break-up. Ecological data provided by those taxa are, however, seldom evaluated.

The size and position of Gondwanaland implies the existence of climato-ecological zonation of the mega-continent. Such zones must have existed between the extremes of oceanic and continental, as well as between cool-temperate and tropical zones.

Depending on the level of conservatism to their native habitats, a range of taxa appear to inhabit the same climato-ecological areas on the separated continents even at present. Examples will be presented from different families of Coleoptera that are indicators of climato-ecological zonation of Gondwanaland. The continuity of former will be shown between South America (Antarctis) New Zealand and Australia, and between Africa and its sister continents and land-masses.

02-033

THE BIOGEOGRAPHY OF WEEVILS ON SOUTHERN OCEAN ISLANDS

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The *Ectemnorhinus*-group of weevils is a monophyletic unit within the Brachycerinae (*sensu* Kuschel), endemic to the South Indian Ocean Province Islands of the sub-Antarctic region, i.e. the Prince Edward Islands, Crozet and Kerguelen archipelagos, and Heard and McDonald Islands. Phylogenetic analysis has demonstrated that the adelphotaxa for the *Ectemnorhinus*-group are the genera *Heterexis* Broun and *Oclandius* Blanchard which occur on the Auckland and Campbell Islands. It is suggested that the weevil faunas of the Crozet and Kerguelen archipelagos were derived following vicariance with East Antarctica and via Antarctica, New Zealand and its associated islands. Sub-Antarctic chironomid taxa show similar relationships, suggesting that the faunas of the South Indian Ocean Province Islands were derived partly from New Zealand. The weevil faunas of the New Zealand sub-Antarctic islands have close relationships with those of New Zealand, and likewise the Falklands fauna, and that of Gough and Tristan da Cunha, appear to be derived from those of South America. Curiously, weevils are absent on both South Georgia and Macquarie Islands. The weevil faunas on all of the sub-Antarctic islands appear to have been affected dramatically by Neogene climatic fluctuations. In the *Ectemnorhinus*-group this can be seen most clearly in a switch from angiosperm to cryptogam feeding, and reduced species richness in the basal, angiosperm-feeding taxa. The cryptogam-feeding genus *Bothrometopus* diversified during glacial periods when angiosperms were absent from the islands. The biogeographic patterns shown by sub-Antarctic weevils argue for a more comprehensive re-assessment of the biogeography of southern ocean islands.

02-035

FLYING THE SOUTHERN HEMISPHERE

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The pioneering work of Lars Brundin in developing an explicit biogeographical method that reconciled the histories of biota and earth (phylogenetics and geology) of the cool temperate southern hemisphere remains very influential. Brundin recovered the orderly pattern of geological fragmentation of a pre-Jurassic Gondwanan supercontinent from the current distributions of certain midges (Chironomidae: Diptera).

Additional southern African-Australian chironomid associations confirm and expand the proposed biogeographical relationships. Elsewhere amongst the Diptera, comparable austral patterns are apparent (although sometimes differing in the relationships inferred for New Zealand) within the Trichoceridae, Tipulidae, Dixidae, Simuliidae, Thaumaleidae, Perissomatidae, several families of Mycetophiloidea, and in the Brachycera in at least the Pelecorhynchidae, Tabanidae and Rhagionidae. Entomologists increasing are comfortable with these ancient vicariant patterns and the ages they imply. However, we may be blinded to alternative faunal linkages between more tropical areas (some of which are no longer "austral" in that they are north of the equator). Patterns of faunal association between northern Australian insects and those of "Asia" were recognized early, and interpreted as due to recent dispersal from Asia (i.e. post-Miocene austro-Asian contact). Increasing evidence from the Chironomidae demonstrates concerted patterns of intra-generic faunal connections between central Africa, Madagascar, southern India and Sri Lanka, parts of south-east Asia and northern Australia. This pattern - Croizat's Indian Ocean baseline - requires no invocation of dispersal as an explainer but is an alternative (warm/eurythermic) vicariant Gondwanan pattern.

This evidence suggests that systematic studies that are restricted to a single region, lacking a wider biogeographical framework, or interpreted within only one austral vicariant pattern, may be deficient.

02-036

THE SYSTEMATICS AND BIOGEOGRAPHY OF THE AUSTROMIRINI (HETEROPTERA: MIRIDAE: ORTHOTYLINAE)
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The Austromirini are defined as a monophyletic tribe of the mirid subfamily Orthotylinae (Heteroptera: Miridae) on the basis of characters of the mesothoracic pleura and the genitalia. The tribe contains 36 genera and is found in all Australian states and territories, New Guinea and Hawaii. Australia is the centre of diversity where 34 genera occur, one of which also occurs in New Guinea. Two Hawaiian endemic genera are assigned to the tribe for the first time. A phylogenetic scheme for the genera has been developed and the biogeographic patterns within Australia will be outlined. The area relationships within Australia will be discussed in terms of current models such as Cracraft (1991). The biogeographic disjunction of the Austromirini between Australia + New Guinea and Hawaii will also be discussed in relation to Pacific Basin biogeography and determining the origins of the Hawaiian mirid fauna (Asquith, 1995)

02-038

PHYLOGENETIC RELATIONSHIPS AND CLADISTIC TEST OF THE EVOLUTIONARY CORRELATION BETWEEN OVIPOSITOR STRUCTURE AND OVIPOSITION HABITS OF *SCOTUSSA* AND *LEIOTETTIX* (ORTHOPTERA: ACRIDIDAE)

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A cladistic analysis of the South American grasshopper genera *Scotussa* and *Leiotettix* was performed to test the monophyly of these genera. Characters from the external morphology, male genitalia and female ovipositor were used in the analysis. The association between the structural change that occurred in the ovipositor valves of *Scotussa* and the functional change of the oviposition habits were tested. Information on oviposition habits and geographic distribution was then mapped on the cladogram, to determine the transformation sequence for performance and shift in the environment. Results confirmed the monophyly of *Leiotettix* and *Scotussa*. They also support the hypothesis of association between the structural change that occurred in the ovipositor valves of *Scotussa* with the functional change in the oviposition habits. However, this association did not seem to be correlated with the adaptive radiation in the genus.

02-037

THRIPS ON THE GREAT SOUTHERN ARK

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Australia, the Great Southern Ark, presumably carried with it a considerable fauna of Thysanoptera when it first separated, 60-40my BP, from the southern continent. Most of this fauna may not have survived subsequent periods of aridity, as the continent moved north through 20 degrees of latitude to its present position. The modern thrips fauna is not well-known, less than half of the presumed 1000 species having been described. Two genera in the basal clade of the Order are evident relicts, associated with Proteaceae in the moister regions of the South West. One, *Dorythrips*, is shared with western South America, and the other, *Cranothrips*, with South Africa. One genus in the most derived clade, *Pseudanaphothrips*, is presumed to be the sister-genus of the largest Neotropical genus, *Frankliniella*. But most of the thrips fauna, like the flora on which it is dependant, appears to be autochthonous and is particularly associated with the eremaeon zone. In contrast, recent field work in northern Australia has produced considerable numbers of species with distributions to the North to India and Taiwan. Presumably these faunal elements postdate the Miocene collision of the Australian plate with the Sunda arc, and result from the wind systems that now blow South from Indonesia in the early months of each year. The effect of this on the concept of an 'Australian fauna' will be discussed.

02-039

CLADISTIC BIOGEOGRAPHY OF ASILOID FLIES (DIPTERA: ASILOIDEA) ON GONDWANALAND FRAGMENTS

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Flies of the family Apioceridae have a disjunct distribution, occurring on each of the southern continents of Australia, South America, Africa and in North America. A cladistic analysis based on 77 features of adult morphology revealed that the Apioceridae as currently conceived is paraphyletic. The subfamily Megascelinae, including the genera *Megascelus* Philippi, *Tongamyia* Stuckenberg and *Neorhaphiomidas* Norris, was transferred to the Mydidae to maintain a monophyletic taxonomy. The genus *Apiocera* Westwood is composed of four monophyletic subgenera, one in each disjunct area of distribution. The relationships of the subgenera of *Apiocera* and the genera of the Megascelinae are examined in relation to the vicariance of Pangaea and the breakup of Gondwanaland.

02-040

THE AUSTRALIAN MOTH FAMILY ANTHELIDAE (LEPIDOPTERA : BOMBYCOIDEA) - COMMENTS ON BIODIVERSITY, BIOGEOGRAPHY, AND PHYLOGENY

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The Anthelidae is a family of about eighty described species of medium to large moths, whose distribution is restricted to Australia and New Guinea. Eight genera are recognised from mainland Australia, and four of these are also found in Tasmania. Only one of these eight Australian genera is found in New Guinea, where there is a ninth genus, which is not found either in Tasmania or on the Australian mainland. The Anthelidae belong to two distinct subfamilies: the Anthelinae, with about seventy five described species in seven genera; and the Munychryiinae, with three species in two genera.

To fully appreciate the biodiversity of the Anthelidae, it is necessary not only to look at the phylogeny of the nine genera. One genus, (*Anthela*), includes the majority of the described species, (sixty), and most of these belong to one of several clearly recognisable species groups. The present distribution of these species groups, and speciation within the groups, is often linked to that of their host-plant genus or family. That, in turn, is the result of the climatic and geological history of the continent of Australia.

Several tentative scenarios of both the biogeography and phylogeny of the family Anthelidae are suggested. Its inclusion here, within the Superfamily Bombycoidea (rather than its separation from the Bombycoidea, and inclusion, with the Lasiocampidae, as the Superfamily Lasiocampoidea), is at present fully justified. Many more years work, will probably be required to fully clarify the biogeography and phylogeny of the family Anthelidae.

02-041

ARE INSECTS AS DIVERSE AS THE PLANTS IN THE CAPE FLORISTIC REGION?

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The Cape Floristic Region is characterized by high plant species diversity (more than 8500 species on 90 000 square km) and a remarkable degree of endemism (69 percent for species). As regards the fauna, the mammals and birds are comparatively species poor with a few endemics while the reptiles and amphibians are species rich with many endemics. This information is not available for the insects because of inadequate comparative sampling, and lack of knowledge about the identity and distribution of species. However, by comparing the number of species in certain well-known groups, such as grasshoppers, butterflies, ants and gall insects from specific areas with those of other regions, it is concluded that the insect diversity is not particularly high. The same appears to be true for the degree of endemism, except in specific taxa. It is speculated that this is probably the result of the low nutrient status of the soil, the sclerophyllous nature of the vegetation, the low levels of detritus on the soil surface and frequent fires.

02-042

PATTERNS OF INSECT DIVERSITY IN AUSTRALIA'S TROPICAL MONTANE RAINFORESTS AND THEIR USE FOR CONSERVATION ASSESSMENT

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The Wet Tropics Zone of Australia is a belt of rainforested mountains about 450km long and 70km wide running from 16° to 19° S along the coast of north Queensland between Cooktown and Townsville. The mountains themselves are geologically old (mostly Mesozoic granites) and, by Australian standards, comparatively high. Some exceed 1600m, making them the second highest mountains in Australia. Their montane zones support many relict organisms which date from the gondwanan connection of Australia with Antarctica. Among insects these include many "tropical" occurrences of taxa otherwise occurring only in southern temperate environments, e.g. migadopine carabids, myerslopiine leafhoppers and peloridiid moss bugs. The region has the richest flora and fauna in Australia and was made a World Heritage Area in 1988.

An intensive survey of several target taxa of forest floor insects has been carried out over the last 15 years. The taxa chosen were those with a high proportion of flightless species which would more strongly reflect the historic patterns of distribution. These taxa were the carabid beetles, the adeliine and coelometopine tenebrionid beetles and the aradid bugs.

Fifteen thousand specimens of 265 species of these taxa were collected from more than 350 localities using pitfall traps, intercept traps, litter extraction, pyrethrum spraying and day/night hand collecting. Mapping and analysis of the distribution records showed that many species have very restricted distributions and that the fauna of different mountain massifs varies radically, both quantitatively and qualitatively. Certain massifs proved preeminent as refugia and centres of diversity. Using the collection data, which had been collected uniformly across the region, a Rare and Restricted Index was calculated for each species. This Index ranged from 1 to 20 and comprised components from five aspects of the data, viz. number of specimens collected, number of times encountered, number of localities encountered, number of sub-zones encountered, and altitudinal restriction. Thus species known from a single specimen from high altitude achieve a score of 20 while those collected many times at many localities at all altitudes score close to zero.

Calculating means or sums of the Indices of all the species on each of 39 individual mountain massifs of the region allows the massifs to be ranked in terms of the relative size, endemism and rarity of their insect faunas. This gives a simple tool to assist decisions regarding the conservation management of different parts of the Wet Tropics World Heritage Area.

02-043

BIODIVERSITY ASSESSMENT USING LEPIDOPTERA — SELF INTEREST OR COLLECTIVE INTEREST?

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At a conservative estimate, there are about 250,000 species of Lepidoptera living today. Their conservation on an individual, species-by-species basis is not a practical option, so the fate of all but a few (other than those favoured, or not threatened by human activities) will depend on the selection and maintenance of suitable habitats. Networks of conservation areas, even when they are planned with a representation goal in mind, are normally based on the analysis of vertebrate distribution patterns, or on vegetation or land classifications. There is, however, no compelling empirical evidence or theoretical reason to expect that networks based on vertebrate data, or other, more remote biodiversity surrogates, will be fully effective, or maximally efficient for the conservation of Lepidoptera (or any other non-focal taxon). Lepidoptera may need action plans in their own right. Alternatively, they may have potential to act as surrogates for other, less well-known elements of biodiversity, through analysis at species or higher-taxon level, or as part of a suite of focal taxa for biodiversity assessment by summation. Finally, they may also help to identify areas of endemism, a largely untested approach to conservation evaluation. The talk will be illustrated with examples of research currently in progress, mainly drawn from work on Papilionoidea and Bombycoidea being carried out at the Biogeography and Conservation Laboratory of The Natural History Museum, London.

Biodiversity of Lepidoptera: background and application. R. de Jong & M. Scoble

02-044

LEPIDOPTERA AS AN 'UMBRELLA GROUP' FOR INSECT CONSERVATION.

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Much of the development of insect conservation has directly involved studies on butterfly species or subspecies, or has been stimulated by these. Butterflies have thereby become the most important 'flagship' group of insects in practical conservation. Species-level focussing may be impracticable for more diverse groups, including many moths. The ecological complexities collectively, manifest by Lepidoptera, together with increasing taxonomic tangibility of many groups and the ease of sampling them, can be used to promote the worth of butterflies and moths as an umbrella group, capitalising on existing public and official sympathy and extending appreciation of insect diversity and ecological values. Opportunities for broad promotion of insect conservation in many parts of the world using Lepidoptera are explored and discussed.

02-046

BIOLOGICAL COLLECTIONS AND BIODIVERSITY
STUDIES OF LEPIDOPTERA
S. Nielsen (Canberra-Australia)

ABSTRACT NOT RECEIVED

02-045

USING TAXONOMIC DATA IN BIODIVERSITY STUDIES ON THE LEPIDOPTERA
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The Lepidoptera, particularly butterflies and large moths, are well represented in insect collections. Basic taxonomic details in digital form now exist for a substantial portion of the Macrolepidoptera.

A taxonomic database of the Geometridae is substantially complete. The mechanics of this project show that it is perfectly possible to produce life-lists of large insect taxa within a relatively short period of time. Such information has been requested frequently by the biological community - particularly in recent years.

Details of the geometrid database will be presented, and use of the information in studies of species richness explained.

02-047

INVASIONS OF LEPIDOPTERA AND THEIR EFFECT ON BIODIVERSITY.
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Invasions are defined and distinguished from migration and artificial introductions. Data are presented on lepidoptera which have successfully invaded the British Isles during the last 100 years. Further data quantify extinctions, and the last sightings of species which have not been recorded for over 25 years.

Many extinctions can be attributed to changes in land use and other factors, rather than to competition with other species of lepidoptera. Examples from plants and mammals show tht invasive species do affect native populations. Data from British lepidoptera are examined to explore whether there is any such link, however indirect, and how invasions relate to indices of diversity.

02-048

EVALUATING THE EFFICIENCY OF TARGET TAXA IN BIODIVERSITY ESTIMATION: MYCALESINE BUTTERFLIES IN MADAGASCAR.

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Madagascar is a global biodiversity hotspot. Its biodiversity is poorly known, and only 6% of its remaining primary forest is protected; even this protected forest is threatened by a spectrum of human-related causes. For efficiency in survey work and conservation strategy, it would be useful if we could estimate biodiversity patterns from knowledge of a well studied group. In the U.K. at least, local hotspots do not always coincide. In Madagascar, however, the primary rainforest biome (mostly still contiguous except at lower elevations) provides a more promising test. Because surviving rainforest organisms there share a common evolutionary theatre and ecological play, there is a much better chance that diversity patterns may show congruence—especially when the target group has undergone an extensive local adaptive radiation, as has happened in one subtribe of satyrine butterflies, the Mycalesina. As a result of recent work on this group, species definitions, phylogenetic relationships and distributions are now better understood than for most other Madagascan invertebrates. To assess the potential of the target taxon approach, patterns for mycalesines are compared with those for other well known rainforest taxa.

02-050

PHYLOGENY OF THE OLD WORLD YPONOMEUTIDAE S.STR. (LEPIDOPTERA). A TOOL FOR THE FORMULATION OF SCENARIOS OF SPECIATION

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The morphology of the *circa* 200 Old World species of the moth family Yponomeutidae has been investigated. The relationships within the Yponomeutidae have been worked out on basis of cladistical analyses of the morphological differentiation among the species. Previous hypotheses concerning relationships among the Yponomeutidae are reviewed and new hypotheses will be presented.

Special reference will be made to the genus *Yponomeuta* Latreille. This palaearctic genus has been studied as a model system for the evolution of insect-plant associations and speciation in phytophagous insects since the early seventies.

Scenario's for processes of speciation within the genus *Yponomeuta* are obtained by superposing characters that might act as isolating mechanisms such as geographical distribution, host plant association and sex pheromone communication onto the independently derived estimate of the phylogenetic relationships among the species.

The scenarios are formulated along with the sequences of character changes dictated by the cladograms presented here. Simultaneously, the restrictions to the processes imposed by the cladograms will be discussed.

02-049

BUTTERFLIES AND PLANTS: A PHYLOGENETIC STUDY

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A database on host plant records from 437 ingroup taxa has been used to test a number of hypotheses on the interaction between butterflies and their host plants using phylogenetic methods. Previous studies suggest that the patterns of association in most insect-plant interactions are more shaped by colonizations, host shifts, and specialization than by cospeciation. Consequently, in this paper we have focused explicitly on the mechanisms behind host shifts and changes in host range.

The ancestral host plant appears to be located within a highly derived Rosid clade, which includes Fabaceae, Urticales, and Rosaceae. Host shifts have been more common between closely related plants than between more distantly related plants. Plant growth form turned out to be an even more conservative aspect of the interaction between butterflies and their host plants than plant phylogeny. However, this is largely explained by a higher probability of major host shifts while feeding on trees than on other growth forms. There was also a strong association between tree-feeding and a large host range, but no support at all for the view of specialization as an evolutionary "dead end". Instead, most lineages appear to have undergone repeated instances of polyphagy, followed by re-specialization. These three last results together suggest a possible general pathway for host shifts, via a polyphagous state on trees.

02-051

SYSTEMATICS AND BIOGEOGRAPHY OF AFRICAN MACARIINI (LEPIDOPTERA: GEOMETRIDAE: ENNOMINAE)

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Revision of the macariine fauna of Africa, Madagascar and Arabia resulted in major changes to the global taxonomy of the group on generic and tribal levels.

A total of 264 species, 93 of which are described as new, are recognized. These species are distributed very unevenly over 15 genera, eight of which are new to science.

Following cladistic analysis of macariine genera on a worldwide basis, a subdivision of the study group into two subtribes, Platypeplina and Macariina, is proposed.

Within the larger genera, numerous monophyletic species-groups are present, many of which exhibit associations with one of the major biomes. It is hypothesized that the two largest genera, *Itame* Hübner and *Chiasmia* Hübner, have speciated extensively in the savanna biome of Africa during the Cenozoic, as their derived species-groups are almost entirely confined to savanna habitats today, whereas many of the less derived groups occur in the older forest biome. The 'modern' species-groups of both genera are also completely absent from Madagascar, which has been separated from the African continent since the late Mesozoic. Based on present-day distributions and phylogenetic information, evidence is presented for a post-Gondwanan origin of Macariini in the Afrotropical region.

02-052

NOTES ON THE CLASSIFICATION OF AMERICAN INCHWORM MOTHS IN THE SUBFAMILY STERRHINAE (LEPIDOPTERA, GEOMETRIDAE).
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The smallest inchworm moths are members of the worldwide subfamily Sterrhinae, and comprise more than 2,378 species in 100 genera. Nearly 1,000 species representing 6 tribes have been described from the Americas.
 Because of their polymorphism and the geographic interests of previous workers, much synonymy and confusion exist. While the status of the 103 North American species has been updated, most of the vast neotropical fauna remains to be worked out. Some genera, such as those of the diurnal tribe Cylopodini seem out of place as currently classified in the subfamily.
 The author comments on the current status of classification and proposes a program to elucidate the true status of these moths by collaboration with workers in Central and South American countries such as Costa Rica (through INBio) and Ecuador.

02-054

R.C.L. PERKINS AND THE FAUNA HAWAIIENSIS: WHAT LONGTERM DATA CAN TELL US ABOUT HAWAIIAN DIVERSITY
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From 1893 to 1897, R.C.L. Perkins conducted a faunal survey of Hawaiian animals that formed the basis for the Fauna Hawaiiensis. This survey, conducted by a single individual who kept substantial field records, and who noted specific sites that can be revisited today, resulted in taxonomic material that provides a valuable basis for comparison 100 years later. In addition, Perkins' notes can be tied to specimen voucher lots, making biological phenomena such as relative abundance, phenology, way of life, and habitat type discernible from his collections. We first focus on the immense impact introduced animals and plants have wreaked on native Hawaiian ecosystems over the past 100 years and more; a fact lamented even by Perkins. We then compare diversity estimates derivable from Perkins' collections with those made during later decades using two model groups; *Megalagrion* damselflies (Odonata) and platynine carabid beetles (Coleoptera). Both groups have been extensively surveyed on all islands in the 1990's, with many of Perkins' collecting sites revisited. Profiles of relative abundance for relatively undeveloped Molokai deviate little from the 1890's to present, indicating that in protected montane areas, populations of native damselflies and carabids have not been impacted by biotic change or habitat destruction. A single potential extinction, the terrestrial breeding *Megalagrion molokaiense* (Perkins), may have been compromised by ants. Conversely, on highly developed Oahu, many rarer taxa, and one previously common carabid, *Colpocaccus tantalus* (Blackburn), have not been observed during the present decade.

02-053

A PRELIMINARY ANALYSIS OF THE PHYLOGENY OF THE POLYOMMATINI (*Polyommatus* GROUP OF GENERA, SENSU ELIOT)
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 The Polyommatini represent a rather homogeneous assemblage of genera, easily enough definable by the presence of a transversally split tergal sclerite (pseudotergum) in the male genitalia and of an extrovertible appendage (henia), in the female sex. Ideally, females take an active role in copulation, by seizing the highly reduced and barely protruding male's suprazonal part of penis by their external genital opening, placed on the tip of the henia. Other additional characters are represented, in the male sex, by a double, digitate, uncus accompanied by a pair of falx-shaped subunci, a U-shaped futura inferior (furca) and fusiform valvae. Males, moreover, normally have battledore shaped androconial scales. Not all these characters, however, are recognizable in all species and particularly some of the apparently least advanced genera may lack one or more of them. Conversely, at least in another non-Polyommatine genus such as *Lampides*, females have a non-extrovertible henia, whereas males do not show any trace of a pseudotergum. This genus is provisionally assumed to represent the sister group of the Polyommatini. Within the main Polyommatine lineage, however, specialization does not proceed linearly towards the acquisition of the most advanced genital structures and many of these acquisitions may well have been developed more than once, in different sub-lineages. In the present paper I will develop a preliminary phylogenetic analysis based on 67 mostly genitalic characters as observed in 42 genus-group taxa. A group of South and Central American genera appear to include the most primitive taxa of the Polyommatine lineage, which might have spread into the Holarctic realm only in comparatively recent times. Some of these more advanced genera may have, in turn, re-invaded South America.

02-055

RADIATION AND PHYLOGENY OF TETRAGNATHID SPIDERS (ARANEAE) IN HAWAII
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 A large radiation of *Tetragnatha* endemic to Hawaii has been recently uncovered, which were previously known only from descriptions of 9 species, described on the basis of specimens collected in the 1890's by R.C.L. Perkins. Over the last few years an additional 19 species of Hawaiian *Tetragnatha* have been described and » 50 new taxa have been collected. This species radiation spans a huge spectrum of colors, shapes, sizes, ecological affinities, and behaviors, but form closely related lineages within the genus *Tetragnatha* based on palpal morphology, cheliceral structure, mating behavior, and molecular data. Many are web-building, with structural modifications of the abdomen that allow concealment within specific microhabitats. Some species have modifications of the cheliceral armature, apparently to allow specialization on specific prey types. However, several groups have abandoned the characteristic web-building behavior of the genus. For example, one entire clade of 16 species, has abandoned web building, with the concomitant development of long spines along the legs and adoption of a vagile, cursorial predatory strategy. In this paper I discuss patterns of speciation in the lineage, their relationship to habitat specialization, and the possible number and source of colonizations involved in the species radiation.

02-056

SYSTEMATICS AND EVOLUTION OF HAWAIIAN FULGOROIDEA (HEMIPTERA)

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With ca. 230 endemic species, the phytophagous Fulgoroidea or planthoppers are among the most significant elements of the native Hawaiian fauna. As predominantly mono- or oligophagous insects they play an important role in nearly all Hawaiian ecosystems. Species of only 2 out of worldwide 18 planthopper families have successfully colonized and subsequently radiated in Hawaii. Based on collections mainly made by Perkins, Kirkaldy, Muir, Giffard, and Swezey, more than 95 % of these species were discovered and described in the first two decades of this century. Recent inventories on all islands and especially studies on the intraspecific communication of selected planthoppers (e.g., cavernicolous taxa of the family Cixiidae) have revealed a much higher degree of diversity leading to an estimate of a total of 350-400 species. However, the taxonomy of this group still reflects the standard of the early century. Currently, on the basis of comparative morphology only few Hawaiian planthopper genera can be regarded as monophyletic, while others are evidently polyphyletic, including *Nesosydne*, the largest genus of Delphacidae in the Pacific which is also reported from areas outside of Hawaii (e.g., Marquesas, Society, and Galápagos Islands). While in Cixiidae apparently 2 independent primary colonizations of yet uncertain origin of the ancestral species have occurred, at least 5 primary invasions equally from an unknown source can be estimated for the Delphacidae. However, as long as the systematics of the Hawaiian planthoppers is not yet based on a phylogenetic analysis, any conclusions on biogeography and evolutionary history of this group may be rather tentative. A modern biosystematic study of the native Hawaiian planthoppers at all biological levels is proposed and will include morphological and molecular systematics, behavioral ecology, and population genetics; the latter especially in populations of cavernicolous taxa and those presumably co-evolved or co-evolving with certain hostplants or plant communities (e.g., a group of ca. 10 apparently closely related morphospecies of *Nesosydne* s. str. with *Acacia* kōa).

02-057

HAWAIIAN PSEUDOCOCCIDAE (HEMIPTERA), A GROUP THAT PERKINS MISSED.

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Among the 16 or so recognized families of Coccoidea, only Pseudococcidae and the small, specialized Halimococcidae are represented in the endemic Hawaiian fauna. Why other large coccoid families failed to establish there is unknown. The endemic Pseudococcidae of Hawaii presently include 31 described species in 13 genera. Ten genera are endemic. Around 40 undescribed endemic mealybug species belonging to both described and undescribed genera also are known. Perkins apparently collected no endemic mealybugs. Kirkaldy in *Fauna Hawaiiensis* listed the "Family Coccidae" (= Coccoidea) as absent from the endemic Hawaiian fauna. At least 5 or 6, possibly more, prehistoric colonizations of Hawaii by mealybugs were required to produce the existing fauna. Most of the endemic genera are so highly specialized that their relationships to extra-Hawaiian forms are obscure. However, some endemic species of *Pseudococcus* appear to be closely related to species in Australia and Pacific islands. This conclusion is based primarily on similarities in male genitalia; secondarily on female morphology. Endemic Hawaiian mealybugs are often cryptic, occupying habitats such as plant galls, rolled leaves, under bark and leaf sheaths of grasses. Those which occupy more exposed locations on foliage or twigs usually are cryptically colored or armed with large spines. These specialized habitats and morphologies appear to have evolved in response to pressure from predators.

02-058

THE PHYLOGENETIC ECOLOGY AND BIOGEOGRAPHY
OF HAWAIIAN *MEGALAGRION* DAMSELFLIES -
A CASE STUDY IN EVOLUTION

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The damselfly genus *Megalagrion* as currently interpreted contains 28 species and subspecies, all endemic to Hawaii. An analysis of external morphological characters in both males and females has permitted a reconstruction of the phylogeny of this group, and the delimitation of major clades within it. This analysis clearly indicates that the presently existent clades within *Megalagrion* were established by at least the time the island of Kauai came into existence approximately 5 million years ago, and that members of these clades have moved sequentially down the chain as newer islands were created. Members of these clades also possess distinctive ecological preferences that are retained as new islands are colonized. These phylogenetically linked ecological traits are useful in a conservation context by allowing the prediction of ecological preferences in species for which this information is currently unknown.

02-059

THE HAWAIIAN DROSOPHILIDAE: A MOLECULAR
PERSPECTIVE.

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There are probably over one thousand species of Drosophilidae distributed in two subgenera in the family that are endemic to the Hawaiian archipelago. The ecological, morphological and behavioral divergence of several of the species groups of Hawaiian Drosophilidae is well documented. In addition, the classical chromosomal analyses of Carson and his colleagues lends a genetic perspective to our understanding of the radiation. More recent molecular analyses can also contribute to our understanding of the group as this talk will demonstrate. Three major areas where molecules have contributed to our understanding of this interesting radiation will be discussed. All are related to a better understanding of phylogeny in the group. The first concerns species level phylogenetic analysis to better understand the biogeographic relationships of these organisms. Molecular phylogenies can be used to examine the patterns of interisland colonization and to give us some idea of the trends involved in the biogeography of the group. Another biogeographic problem that can be addressed using molecular analysis concerns the founding of the entire group on the archipelago. Using molecules as a source of characters a higher level phylogeny of Hawaiian taxa and continental taxa can address this question. The second area that this talk will address is the issue of ecological and morphological radiation. Higher level phylogenies can be used to understand patterns of cladogenesis in the group and to examine the correlation of morphological and ecological change in the group. The final area that molecular analysis can be of use is in the diagnosis of species. Using molecular characters the problem of species designation of *Drosophila silvestris* and *Drosophila heteroneura* on the Big Island of Hawaii will be examined.

02-060

ADAPTIVE RADIATION IN THE HAWAIIAN DROSOPHILA:
ECOLOGICAL AND REPRODUCTIVE CHARACTER ANALYSES

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The entomologist R.C.L. Perkins pioneered observations of the breeding site ecology of the endemic Hawaiian Drosophilidae, a renowned group of flies that has undergone explosive speciation and adaptive radiation into a wide variety of breeding niches. Females of the various species groups and subgroups oviposit their eggs in fungi, decaying flowers, fruits, leaves, stems, bark, tree fluxes, or even spiders' eggs, in a few cases. Varied selective forces in these alternative niches have molded female reproductive characters and strategies into diverse outcomes; some species mature and oviposit only one egg at a time, whereas others oviposit hundreds.

Recent molecular phylogenies of Hawaiian drosophilid species based on nuclear and mitochondrial DNA sequences permit phylogenetic tracing of the evolutionary shifts in female behavioral and morphological aspects of reproduction, and reconstruction of the history of the remarkable adaptive radiation of this fauna. Phylogenetic character analyses to be presented focus on aspects of female ovipositional choice, and ovarian, ovipositor and egg chorion morphology. The correlations among behavioral, morphological and ecological traits, and adaptive aspects of character evolution in the Hawaiian drosophilid fauna will be analyzed.

02-062

PHYLOGEOGRAPHY OF PLANTHOPPERS
(HEMIPTERA) AND TEPHRITIDS (DIPTERA) ON THE
SILVERSWORD ALLIANCE: PARALLEL SPECIATION
OR CO-EVOLUTION?

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Insect species in several lineages feed exclusively on plant species in the Hawaiian silversword alliance (Asteraceae). These lineages include species of delphacid (*Nesosydne*) planthoppers and tephritid (*Trupanea*) flies. For both lineages, phylogenies based on molecular data, are presented for insect species collected from plant species within the Hawaiian silversword alliance. The patterns of radiation for the insects are compared to that generated for their host plants in the alliance, also based on molecular data (from B. G. Baldwin and R.H. Robichaux 1995). Co-speciation is found to be significant between planthoppers and their hosts, but less so for tephritids. I also examined the role that hybridization between members of the Hawaiian silversword may play in the diversification of plant feeding insects. Host plant hybridization may create "resource sinks," such that insect species from both parental plant species are found on hybrids, or alternatively, the hybrids may serve as "bridges," by which insects of one parental plant species move to hybrids and then onto new hosts. These hypotheses are explored by examining the relationships of insect species found on hybrid and parental plant species.

02-061

THE HAWAIIAN DROSOPHILIDAE: WHERE DO WE
GO FROM HERE?

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More than three decades of research on the evolutionary biology of Hawaiian Drosophilidae have made significant contributions to the understanding of evolutionary processes on islands. While the first ten years of the project involved field studies to determine the extent of the biodiversity of this group, the last 20 years have focused on unravelling genetic mechanisms of speciation primarily on one group of species, the picture-winged species group. Detailed morphological, cytological, biochemical, developmental, and molecular studies have been conducted on this group of about 100 species by more than 50 researchers from the U.S. and abroad.

We are just beginning to investigate the biosystematics of the other groups in the Hawaiian Drosophilidae. Detailed morphological analyses of the modified mouthparts and modified tarsus groups are in the early stages; ecological, developmental, and molecular studies of these groups are also proposed. While the picture-winged species have provided excellent subjects for evolutionary studies, the remaining species in this remarkable fauna will provide important insights into the evolution of some of the bizarre secondary sexual structures observed in dipteran species. Scanning micrographs will show the elaborate development of these structures and their possible sensory role in the courtship displays of the males.

02-063

DISPERSAL AND VICARIANCE IN HAWAIIAN PLATYNINE
CARABID BEETLES (COLEOPTERA)

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The Hawaiian Islands support a substantial native carabid beetle fauna, with 129 of the approximately 300 native species comprising a monophyletic radiation within Tribe Platynini. Overall, 125 of these 129 species are single island endemics; all 24 species from Kauai, 33 of 34 species from Oahu, 17 of 21 from Molokai, 3 of 5 from Lanai, 13 of 17 from West Maui, 31 of 35 from Haleakala, and 4 of 6 from Big Island. Cladistic analysis of the 129 native species plus 41 outgroup taxa, based on 204 morphological characters, indicates that island colonization within the chain is almost completely progressive, with the origin of the fauna on Kauai. Outgroup area relationships are somewhat ambiguous, with the Australian *Notagonum submetallicum* White patristically closest to the Hawaiian radiation. Extensive endemism on the separate volcanoes comprising Maui Nui allows comparison of vicariant patterns among Molokai, West Maui and Haleakala. Five resolvable three-species triplets occupy the three islands; three exhibit a Molokai-West Maui sister relationship, and two support West Maui and Haleakala as most closely related. Four other species are widespread across the three islands. These patterns are analyzed in light of elevational range of known habitat, relative abundance, phylogeny, and way of life. Elevational range and relative abundance correlate best with the type of vicariant pattern observed, whereas way of life correlates best with presence or absence of speciation. The colonization of Big Island is presented as a model of previous island colonizations. The six Hawaii species are derived from at least five colonization events, three involving winged progenitors, two involving vestigially winged progenitors, and all from a source on adjacent Maui Nui.

02-064

INSECTS IN A CHANGING WORLD.

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I. Distribution. Significant northward movement of several southern insect species was observed in the last years in different parts of Europe, by average speed of 20-50 km/year. In some cases by "jumping dispersion", insects could reach 700 km in a very short time. In a lesser extent some species showed south east extension of their distribution. What could be explained by the relatively cold summers, observed in this region in the years with mild winters.

II. Population dynamics. New outbreaks of thermophilous insects were observed in different countries, such as *Dociostaurus maroccanus* in Hungary. The new outbreaks of *Ceratitis capitata*, *Helicoverpa armigera* in South Europe, were followed by northward migrations of these pests in Europe.

III. New introductions. In Europe several important new introduced pest species were observed, such as *Diabrotica virgifera virgifera* in Serbia, two *Ragoletis* species in Italy and Switzerland, etc.

IV. Phenology. In some countries after mild winters, warmer spring was observed, with subsequent earlier beginning of the phenological changes on the insects, but the changes are very slow, about 7-10 days/100 years. In contrary, in Central Europe after milder winters colder springs were observed, with later appearance of insects.

The northward expansion, the new outbreaks, introductions and northward migrations of several thermophilous insect species could be explained with long period of years with mild winters, and with hot summers.

02-066

INFLUENCES OF CLIMATE FLUCTUATIONS ON INSECT DIVERSITY PATTERNS DETECTED BY LONG-TERM LIGHT TRAPPING

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It is important to monitor the effects of climate change on communities of living organisms. The insects are suitable bioindicators of the fluctuations of the climatic variables due to their rapid responsibility.

The authors have studied the transformations and fluctuation patterns of insect diversity depending on climatic variations in Hungary during the last 34 years. Long-term yearly light trap captures of macrolepidoptera (1962-95), predatory brown lacewing and antlion (Neuroptera: Hemerobiidae, Myrmeleontidae) (1981-95) assemblages were used for analyses. The light trap network stations were operated inside forested and agricultural habitats in both lowland and highland regions.

The monthly climatic characteristics (temperature and precipitation variables) and various drought indices were calculated from data sets of the standard network of Hungarian Meteorological Service.

Time series analytical methods were applied to characterize the changes in the patterns and to detect the influences of climate variations. Various indices and functions (species richness, H', alpha, Q, etc.) served for description of diversity patterns.

02-065

1945-1995: FIFTY YEARS OF INCIDENTAL INSECT PEST INTRODUCTION TO ITALY

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More than one hundred exotic insect pests have been introduced to Italy in the years between 1945-95. They are mainly pests of ornamentals, woody plants and Citrus. Some of them are pests of medical and domestic importance (i.e. *Aedes albopictus* (Skuse)). The three orders accounting for most of the introductions are the Homoptera (70% of the total number of introduced species), Coleoptera (10%), Lepidoptera (7%). The majority of introduced species have come from North America (26%), Asia (25%), Central and South America (12%), Africa (10%) and Australia (7%). Some introduced species failed acclimatization because of adverse climatic factors or extermination of the focus (i.e. *Demyrsus meleoides* Pascoe on *Cycas*, *Limacoccus brasiliensis* (Hempel) on *Arecastrum*). Many introduced species persist only in greenhouse: (i.e. *Pseudococcus microcirculus* McKenzie on orchids, *Rhizoecus dianthi* Green and *R. saintpauliae* Williams on ornamentals). Several species have succeeded in acclimatizing and nowadays are widely distributed throughout Italy but with low population densities (i.e. *Diaspidiotus osborni* New. & Cock. on plane, *Dasineura gleditsiae* (Osten Sacken) on *Gleditsia*). In other cases the introduced and acclimatized pest has not spread from the first focus noticed but persists as a local pest (i.e. *Epithrix hirtipennis* (Melsh.) on tobacco *Gonipterus scutellatus* Gyllenhal on Eucalyptus). Many species have succeeded in acclimatizing and in spreading and are now largely distributed at high population densities throughout Italy (i.e. *Ceroplastes japonicus* Green *Hyphantria cunea* Drury, *Frankliniella occidentalis* (Pergande)). In some cases Italy has been the first noticed focus of an exotic pest in Europe. From this first focus the pest has expanded throughout Italy and towards neighbouring countries (i.e. *Corythuca ciliata* Say on plane, *Metcalfa pruinosa* Say *Paratopopoda robinella* Clemens on locust-tree).

02-067

NORTH AMERICAN FOREST INSECTS AND CLIMATE CHANGE: POTENTIAL CHANGES IN GEOGRAPHICAL DISTRIBUTION OF DEFOLIATOR SPECIES

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The geographical ranges and spatial extent of forest defoliator outbreaks are likely to shift with climate change. We investigate potential changes in spatial distribution of outbreaks of the western spruce budworm, *Choristoneura occidentalis* Freeman, and the gypsy moth, *Lymantria dispar* (L.), in the United States using maps of historical defoliation, climate, and forest composition in a geographic information system. Maps of defoliation frequency were assembled from historical aerial reconnaissance data. Maps of monthly means of daily temperature and of monthly precipitation averaged over 30 years were developed using an interpolation technique. Relationships between defoliation status and environmental variables were modeled with a linear discriminant function. Five climate change scenarios were investigated: an increase of 2° C, a 2° increase with an increase of 0.5 mm per day in precipitation, a 2° increase with an equivalent decrease in precipitation, and equilibrium projections of temperature and precipitation by two general circulation models (GCMs) at 2×CO₂.

With an increase in temperature alone, the projected defoliated area decreased relative to ambient conditions for the budworm and increased slightly for the gypsy moth. With an increase in temperature and precipitation, the defoliated area increased for both species. Conversely, the defoliated area decreased for both when temperature increased and precipitation decreased. Results for the GCM scenarios contrasted sharply.

02-068

TEMPORAL AND SPATIAL FLUCTUATION PATTERNS OF MOTHS UNDER CLIMATIC CHANGE IN HUNGARY

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Recently some characteristic trends in climate pattern have been detected in Hungary (e.g. series of mild winters and decreasing amount of rainfall with frequent droughts). The authors have analysed the effects of these trends and climatic variables on temporal and spatial fluctuation patterns of moths. The macrolepidoptera data sets (1962-95) were based on the catches of forestry light-trap network. The total yearly captures were applied in order to characterize the fluctuation pattern-types of moths. Monthly and yearly values of precipitation, mean air temperature, water-table level and drought indices were calculated from data given by the nearest meteorological station to each trap. The periodicity of population peaks and outbreaks, synchrony between fluctuation patterns in different localities and regions, and long-term trends in patterns of defoliators and other moths were studied by the methods of time series analysis. The spatial changes of abundances probably caused by climate variation were also investigated. It was found that the drought periods showed significant influences on the fluctuation pattern of moths.

02-070

THE OCCURRENCE OF *PANONYCHUS CITRI* (McGREGOR) (ACARI: TETRANYCHIDAE) IN NORTHERN ITALY: DISTRIBUTION, HOST RANGE AND PHENOLOGY

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In the late 80ties, symptoms linked to spider mite feeding were observed on ornamental plants, in particular *Prunus laurocerasus*, in Padova (Veneto, Northern Italy). Surprisingly, the symptoms were caused by *Panonychus citri* (McGregor) a tetranychid well-known as one of the major pests of *Citrus* in the world and widespread in the Mediterranean area. Previously, *P. citri* had been recorded only in Southern Italy, especially on *Citrus* species.

Laboratory studies on a population collected on *P. laurocerasus* in Padova showed different rates of developmental success on *Prunus laurocerasus*, *Citrus aurantium*, *Poncirus trifoliata* e *Pyrus serotina*. In particular, the highest values were reached on *P. laurocerasus*. Developmental times were also shorter on *P. laurocerasus* than on *P. trifoliata* e *C. aurantium*. Fecundity was higher on *P. serotina* and intermediate on *P. laurocerasus*.

Field observations, carried out in different sites and on *P. laurocerasus*, show that *P. citri* populations reach higher densities in early summer. Some weeks later population densities dramatically decrease. The impact of predators does not appear important. A new population increase in late summer is seldom observed.

02-069

INVASION OF NORTH AMERICAN FORESTS BY INTRODUCED PESTS: ECOLOGICAL PROCESSES

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The number of introduced organisms in North America has increased markedly in the past several decades. The effects of introduced organisms on ecosystem properties appear to be at least as great as the effects of climate change and air pollution. Among these, insects and diseases have had known detrimental effects and generally appear more dramatic in agricultural or intensively managed areas than forested lands. The effects of introduced insects and diseases are complex and likely as severe as the effects of plants, but they may be more subtle, and hence more insidious. The extent to which introduced organisms influence functional relationships and ecosystem - wide processes is poorly understood. The nature of ecological impacts of an introduced organism depends upon its ecological role, and exotic organisms occupy a variety of ecological niches. Furthermore, insect and disease pest problems are not simply remedied, and can create biological and management dilemmas. Among the examples to illustrate the potential and actual effects of introduced insects and diseases are: gypsy moth (*Lymantria dispar.*), balsam woolly aphid (*Adelges piceae*), pitch canker disease (*Fusarium moniliforme*), and white pine blister rust (*Cronartium ribicola*).

02-071

EFFECTS OF CLIMATIC VARIABLES ON LONG-TERM FLUCTUATION PATTERNS OF GROUND BEETLES (COL., CARABIDAE) IN HUNGARY

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The carabid beetles are important predatory insects in agricultural habitats. Their population size expresses frequently stronger fluctuations. These results produced by using pitfall trapping which is the best method to follow the changes in carabid population dynamics. However there are only few long-term fluctuation pattern analysis of ground beetles.

We used long-term data sets for analysis of flight activity fluctuation patterns of some important carabid species based on collections of Hungarian agricultural light trap network. We selected three carabid species for the analyses which showed the highest total yearly individuals captured in the most light trap sites. These species were the *Clivina fossor*, *Harpalus froelichii*, and *H. griseus*. The populations of *C. fossor* prefer mainly wet habitats, but the two *Harpalus* ground beetles are rather xerophilous spp.

The aims of this study were to show any influence of climate variations on the long-term fluctuation patterns of carabids. The yearly total number of specimens captured by local light traps were applied for the analysis. In the time series analysis we investigated the carabid data set characteristics (periodicity, trends, synchrony between sites and between species) and the effects of climate variables (air temperature, amount of precipitation, drought indices, weather front frequency) on the flight activity fluctuation patterns.

02-072

THE CENTERS OF HOVERFLIES (DIPTERA: SYRPHIDAE) DIVERSITY IN SERBIA

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The Syrphidae, known as hoverflies or flowerflies are an attractive and heterogeneous group of day flying insects and include many distinctive species. These two-winged flies are characterized by diversity, adaptability and a high degree of adaptational radiation.

Investigations of syrphids fauna in Serbia, shown the considerable species-richness of this region. There has been established an account of about 400 hoverflies species until now. Extremely complex processes in formation of the fauna on the Balkan Peninsula during past geological periods resulted in appearance of many different faunistic elements in Serbia. Therefore, in the same region there is a mixture of the species that originated from distinct and distant biogeographical areas.

This paper points out some of important centers of hoverflies diversity in Serbia. The western and eastern parts of Serbia differ in types of refuges and relics due to geographical position (different in climatic, geomorphologic and historical-geological aspects). In the east, limestone gorges (i.g., gorge of Lazareva reka, Sićevačka, Jelašnička) and canyon valleys, since the Tertiary up to the present time, are the most typical refuges of the Tertiary relics and endemorelics. Their specific features provide the possibilities of preservation and living of the zoogeographically different types of species. Especially, the gorges with west-eastern direction offer a shelter for many species with different ecological requirements. To the contrary, the high mountains on the west (e.g., Kopaonik) are richer in Dinaric endemic and northeuropean and centraleuropean boreo-montane species. The third area, mountain Šarplanina, that lie in the south, contains preglacial relicts, besides Dinaric endemic.

02-074

BIOLOGY AND WORLDWIDE DISTRIBUTION OF SOME WEEVIL GENERA (COLEOPTERA: CURCULIONOIDEA)

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Some weevil groups (Coleoptera: Curculionidae) do occupy a narrow range only while others are widely distributed throughout all continents. Biology and host plant preferences of the latter group have been analysed and compared with the distribution patterns.

Recent research indicates, that some genera of the old family Apionidae are occurring in Europe and/or North America as well as in South Africa or Australia on host plants of the same plant family. Examples are *Taenapion* (Urticaceae), *Pseudapion* (Malvaceae) and the *Ixapion*-complex (Rutaceae). In addition some genera of Apionidae with a wide distribution are restricted to Fabaceae.

Different observations have been made in some groups of Curculionidae. In the Rhynchaeninae the genus *Rhynchophus* lives on Rosaceae in Europe. In Australia the host plants belong to the Mimosaceae. A similar pattern is shown by the related genus *Rhynchaenus* occurring on Fagaceae, Myricaceae, Salicaceae, Caprifoliaceae and Betulaceae in Europe and North America. In South Africa several species have been found on *Grewia* (Tiliaceae) and on Rubiaceae. In India it is known to damage *Mangifera* trees (Anacardiaceae), and it lives on *Brachychiton* (Sterculiaceae) in Australia. Remarkably this group is distributed in all continents except South America.

This may indicate that at the time of the separation of the continents some genera of Apionidae had already been adapted to their present host plant families and have remained relatively constant morphologically and ecologically since.

On the contrary the Rhynchaeninae appear to be quite similar morphologically today, but must have undergone considerable ecological evolution since the continents have separated. Possibly this group started to radiate across the ancient supercontinent after separation of South America only.

02-073

ARE BRAZILIAN SPECIES OF *ELYTROSphaera* (COL., CHRYSOMELIDAE), AN APTEROUS GENUS, THREATENED OF EXTINCTION?J. Vasconcellos-Neto, P. Jolivet¹

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The Neotropical genus *Elytrosphaera* is composed of several apterous montane species. The subgenus *Elytrosphaera* s. str. occurs at altitudes varying from 800 to 2000 m mainly on the Brazilian plateau (8 species), and Yungas del Palmar, Bolivia (one species). The Brazilian species are threatened with extinction due their life style and their distribution over areas with high human density with agricultural and industrial activities. These species are poorly known and only the biology of *E. xanthopyga* has been described by P. Jolivet. The adult and larvae feed on leaves of the shrub *Adenostema brasilianum* (Asteraceae) in primary forest at an altitude of 900 to 1000 m at Viçosa-MG.

Data from museum specimens collected during the last 60 years show that adult beetles appear only at the end of spring and beginning of summer, and apparently diapause in the soil during the rest of the year. Since these species are apterous they do not recolonized forest fragments. Fire and deforestation are the mainly factors limiting *Elytrosphaera* populations.

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02-075

ZOOGEOGRAPHY AND BIODIVERSITY OF SYRPHIDAE (DIPTERA) IN EASTERN AFRICA.

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The zoogeography and biodiversity of the hoverfly fauna (Diptera, Syrphidae) of eastern Africa (i.e. Kenya, Tanzania, and Uganda) is hereby discussed based on a database compiled at the National Museums of Kenya and linked with a GIS system. The database contains approximately 3000 block records and is based on literature references and on collection specimens from National Museums of Kenya (Nairobi, Kenya), Natural History Museum (London, UK.), Natal Museum (Pietermaritzburg, South Africa) and Koninklijk Museum voor Midden-Afrika (Tervuren, Belgium). Total number of syrphids in the region is 219 with 171 found in Kenya, 123 in Tanzania and 127 in Uganda. Of these, 70 species are found in all three countries while respectively 46, 27 and 14 are reported for Kenya, Tanzania and Uganda only. The bias towards Kenya is mainly the result of more intensive sampling efforts in this country. Kenya shows a higher overlap with either Tanzania or Uganda than the latter two have with each other. This was looked at as a reflection of the more extensive border line shared by Kenya with the other two countries. There are 44 species endemic to East Africa, 4 of these occur exclusively in Uganda, 10 in Kenya and 11 in Tanzania. Looking at the specimens/country size ratio, all countries have a more or less similar rate of endemism. Analysing the occurrence of Syrphidae at a generic level, most genera show the same tendencies as the ones observed for the family as a whole, with a few exceptions. Although coverage of the region is patchy and the number of block records is limited, some general zoogeographical and ecological tendencies can be observed which are briefly discussed.

02-076

ANALYSIS OF THE BIODIVERSITY OF SYRPHIDAE (DIPTERA) IN DRY TROPICAL RAINFOREST IN KENYA.

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Between 1994 and 1995 Syrphidae were collected at Kakamega rainforest. This forest is situated in the western province of Kenya, and once formed part of a large tropical rain forest belt stretching all the way to Zaire. However, Kakamega forest has been isolated from the main forest for several decennia and forms the easternmost relict of dry guineo-congolian rain forest. It is also the only relict of this vegetation type in Kenya and most of its fauna and flora is unique to the country. Syrphidae were collected mainly by hand sweeping during six successive visits at roughly two monthly intervals. In total, 65 species were found of which 11 were new to Kenya. An additional 9 species were reported from Kakamega forest in literature or presented in older collections. After comparison with other syrphid data for eastern Africa (i.e. Uganda, Kenya and Tanzania) 18 species seem to be unique within Kenya. Of these, 12 show a zoogeographical link with Ugandan forests and represent relicts of the guineo-congolian forest belt, being mainly distributed in Central African and tropical West African countries. Species composition between different habitats within the forest (largely undisturbed and disturbed forest, glades, cleared bushland) and with areas outside the forest are presented with the forest areas showing the largest diversity. The fauna outside the forest boundaries is relatively very poor compared to the species composition within the forest area. Seasonal differences in occurrence were also recorded.

02-078

BEETLES LYCIDAE (COLEOPTERA) OF UKRAINE.

P. Sheshurak

Today 8 species of Lycidae are registered in Ukraine: *Lopherus rubens* Gyll., *Dictyoptera aurora* Hbst., *D. superba* Motsch., *D. nigrorubra* DeG., *Platycis minuta* F., *P. cosnardi* Chev., *Xylobanellus erythroptera* Bdi., *Lygistopterus sanguineus* L. From them only *L. sanguineus* is general, the rest of them are rare. *P. cosnardi* is known only from the Carpathians, the rest can be met in Wooded District; *L. sanguineus* penetrates into the Steppe Zone along the valley of the rivers and can be met in the Crimea too.

All species keep wooded biotopes with enough humidity. In hernigov region the larvas of *L. sanguineus* were noticed in rotten wood. Imagos were met most of all feeding on Umbelliferae or on the trunks of dead trees (*Betula*, *Populus tremula*). *D. aurora* was found on the rotten stump, *P. minuta* were found feeding on Umbelliferae. Beetles are active in day time. Females laid eggs in the crack of the bark or in the hole of ways of various xylophagous.

02-077

AREA TYPES OF HIGH ALTITUDE WEEVIL SPECIES FROM YUGOSLAVIA (COLEOPTERA - CURCULIONIDAE)

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The ecosystems above the timber line at the high mountains of Balkan peninsula are inhabited with a rich fauna of weevils composed by the species from genera *Otiorhynchus*, *Tropiphorus*, *Alophus*, *Plinthus* and *Trachyploeus*.

Two types of weevil communities could be recognise in the high altitude zone on mountains in Yugoslavia: communities at a top of the mountains and around the snow spots at elevations above 2000 m; and communities distributed in different types of mountain meadows and pastures in the wide range of 1700-2100 m above sea level.

The insects from the first group are subnival species and the distribution of the appropriate species is restricted only on the one particular mountain or at geologically and historically very close mountains (southern dinarian, prokletian or scardic mountains). Between different mountain complexes there is a noticeable zoogeographical vicariance. The insects from high mountain meadows have two different types of areas. Most of them are distributed on all mountains from dinaric or scardic mountain complexes, respectively. Sometimes intraspecific variation between populations from different mountains within the same complexes can be observed. The most important factors for their evolutionary divergence were the Pleistocene climatic fluctuations. On the other hand, some species are with wide distribution, sometimes extrazonally but, always in open habitats with degraded vegetation. These species are most probably prediluvian origin and relicts of xerotherm (oromediterranean) communities that were destroyed during glatiations.

02-079

UPDATING OF THE CHECK-LIST OF APHIDS (HOM. APHIDIDAE) IN BELGIUM

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The only complete check-list of aphids (Hom. Aphididae, sensu Pemaudière and Stroyan) in Belgium was published in 1906. In 1956 a new partial check-list was published (related to the Aphididae s.s.). Owing to the changes which have occurred in nomenclature of the aphids, neither of these two check-lists are useful nowadays for non-specialized entomologists and also various dispersed citations have been recorded.

We have revised all the publications on aphids which refer to Belgium and we have prepared a new bibliographical check-list. The known Belgian aphid fauna is made up of 234 species, almost half of them have not been cited since 1945 and many are known from citations prior to 1920. Moreover, only four species have been cited for the first time in this country since 1961.

New aphid fauna studies in Belgium are needed because 1) due to the environmental changes it is very possible that many species cited are not now to be found there, and 2) many other species (known in the north of France, north of Germany, The Netherlands and even Denmark) should populate Belgium.

02-080

ANOTHER NORTH AMERICAN APHID (HOM. APHIDIDAE) ON EUROPE

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News on the presence of insects far from their natural areas of distribution is becoming more and more frequent due to their introduction by humans.

We have captured another North American species: *Myzocallis* (*Lineomyzocallis*) *occulta* Richards for the first time in Europe, limited until now to a few small areas of Canada and the United States and living on *Quercus rubra* which in Europe is grown in parks and gardens.

The subgenus *Lineomyzocallis* is nearctic. This new introduced species was found in a city park in Pontevedra (Galicia, north west Spain) on red oak and mixed with *M. (L.) walshii*, which is already known in France and we have previously cited in that Spanish city.

02-081

TERRESTRIAL INSECTS OF THE PÁLAVA BIOSPHERE RESERVE OF UNESCO

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The Pálava Biosphere Reserve lies in the southern part of the historical territory of Moravia (Czech Republic). The planned extended concept of the Pálava B.R. will cover about 250 sq.km. Three information sources were utilized for compiling the prodrome of insects (prepared in the framework of the prodrome of macroinvertebrates): (1) results of several monitoring programmes implemented in the study area during the past 25 years, (2) data from several dozens of institutional and private collections, (3) records published in about 200 faunistic, ecological and taxonomic papers. In cooperation with more than 40 specialists the comments to all orders of terrestrial (free-living) insects are given.

The research project has resulted in the registration of 9,439 insect species (i.e. about 40 % of the insect fauna known in the Czech Republic). Just from the study area, 99 valid species have been described. Special attention was paid to the stenoeic species confined to xerothermic stands or floodplain forests as widely receding ecosystems. Many species of Pontic, Mediterranean and Pannonian origin reach the northern limit of their range in the Pálava B.R. Data from some long-term monitoring programmes confirmed the extinction of about 20 taxa in the area, additional more than 270 taxa are suggested to be included in different categories (critically endangered, endangered and vulnerable) of species conservation. The condensed information may be used for completing the natural characteristics of the study area, for launching programmes monitoring the selected insect assemblages or taxocoenoses, for objectively assessing the risks connected with human activities in this area and, at the same time, for improving the protection of valuable ecosystems and rare species.

02-082

BIODIVERSITY AND BIOGEOGRAPHY OF CARABIDAE AND TENEBRIONIDAE (COL.) ON THE SOUTH AEGEAN ISLAND ARC

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The south Aegean island arc is well known for its richness in endemic plant and animal taxa. In fact, it is an island landbridge connecting the coast of mainland Greece with the southern shores of Asia Minor, with Crete, Rodos, Karpathos and Kythira being the largest islands with high mountains (1200 to 2500 m except Kythira), many gorges and some permanent water. There are no large plains throughout the islands, except the Mesara plain in central Crete. Due to the varied surface relief and its geographic position, the whole area has diverse climatic conditions, ranging from continental (high mountains of Crete) to mediterranean desert climate (southeast Crete, Kasos, satellite islands etc.).

In order to correlate events from the geological past related to the complex history of the Aegean islands with the present situation and to explain the present faunal composition and origin of the same area, we studied for the past ten years the ground beetle faunas of the south Aegean islands through extensive collecting on the major islands of the arc.

The biodiversity of Carabidae and Tenebrionidae is presented using faunal element and species distribution analysis. The degree of endemism at the specific as well as subspecific level is also discussed. Comparisons between islands revealed extensive faunal dissimilarities, showing more than one possible ways of formation of the south Aegean beetle fauna. The influence of the surrounding areas is also considered.

Cluster analysis using presence-absence data matrices of both families, revealed interesting patterns concerning the zoogeography of the area. We obtained similar results using faunal element categories rather than species in percentage matrices. Three island groups with high degree of internal similarity resulted from the analysis. One group consists of the islands on the west side of Crete (Kythira and Antikythira), a second group was formed by the islands on the east side (Kasos, Karpathos and Rodos) while Crete and its surrounding satellite islands formed the third group. The whole area shows no faunal evidence of common geological evolution after the Pliocene.

02-083

DROSOPHILIDS AND OTHER INSECTS ASSOCIATED WITH PARAHANCORNIA AMAPA FRUITS DISPERSED ON THE AMAZONIAN FOREST FLOOR.

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This community, showed a high species richness but the community structure was marked by a low equitability among its components. *Drosophila* was the predominant group among the insects. A gradual dominance alteration was detected in the *Drosophila* group, with *D. malerkotliana* becoming progressively more abundant all along the period. *D. malerkotliana* was mainly associated with the fruits at the beginning of fermentation and presented a high concentration of only one yeast species, *Kloeckera apiculata*. *Drosophila malerkotliana* was consistently the first species to occupy the fruits on day before the others. The other *Drosophila* species presented a broader diversification in using yeasts and they used fruits in later decomposition stages. All *Drosophila* distribution was affected by the weight of the fruits, pointing to a higher concentration of flies on heavier fruits and to a total exclusion in those fruits below the median weight. Life strategies indicated *D. malerkotliana* and the subgroup species *willstoni* as being fast species and *D. sturtevantii* appeared to be a slow species. *D. sturtevantii* stood out in relation to oviposition patterns, development period and longevity. The increasing success of *D. malerkotliana* observed during these three years of studies corresponded with the recent expansion of the species in the Amazon region and in the Neotropical region as a whole. This and other studies indicate a combination between the species characteristics and modifications occurred in their habitat, favouring their expansion and the decrease in their guild diversity.

02-084

CATERPILLAR SEASONALITY IN CENTRAL BRAZILIAN CERRADO

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The work was carried out in three 1 ha block of cerrado *sensu stricto* near Brasília during five years. The climate is highly seasonal with a marked wet (October-March) and dry (April-September) seasons. A total of 10,800 host plants were examined and on 15% we encountered externally feeding caterpillars. They were reared under laboratory conditions. A monthly abundance for all species of Lepidoptera was compiled and all data of pupation was registered. The caterpillar abundance was variable. It increased sharply in May and remained high until July, at which time declined sharply until the lowest abundance in early-wet season (October) and then increased slightly between December and January. The lowest caterpillar abundance coincided with the driest period, during which the host plant are leafless or with senescent leaves (August-September), but also when the majority of cerrado plants put out their new leaf crop (September-October). The caterpillar community appears to be severely depressed due to the stress of the late-dry season, food shortage and, the onset of heavy rains. Also the small size of caterpillars, after the onset of rains make them inconspicuous in censuses. Two hypothesis were proposed to explain the highest abundance at the early dry season: to avoid consumption of the well defended new leaves and to escape from predators. We successfully reared 247 lepidopteran species (31 families) of which 86.6% occurred at low density (less than 12 times). The duration of the pupal stage and its variance increased in the end of the wet season. They tended to be shorter at the beginning of the dry season, increasing during the dry season and sharply decreasing between the end of dry season and the start of wet season. Some caterpillar species are present on food plants throughout the year (*Isognatus caricae* - Sphingidae) whereas others occur at certain times, either in dry (*Megalopyge albicollis* - Megalopygidae) or wet season (*Siderone marthesia* - Nymphalidae). Emphasis in this work was on the detection of pattern which is a necessary precursor to other studies.

02-086

ENRICHING OUR KNOWLEDGE ON THE GREEK APHIDOFAUNA BY SETTING UP A NETWORK OF ROTHAMSTED TYPE SUCTION TRAPS

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The knowledge of the Greek aphidofauna until 1992 was very poorly known, since there had not been any systematic study performed. Until then 140 different aphid species had been recorded. In 1992 a network of four "Rothamsted", 12.2m high, suction traps were set up in Thessaloniki, Velesino-Thessalia, Kopais-Voiotia and Koroivos-Ilia respectively. During the following four consecutive years a large number of alates were caught in the traps. In total, more than 175 taxa, not recorded previously in Greece, were caught. Most of them were identified to the species level. In Thessaloniki 107 different taxa were collected, in Velesino 139 and in Kopais 145. The trap in Koroivos was not in operation during the above period. For all the species detailed weekly, and in some cases daily, captures have been kept. Although the highest numbers of alatae were observed during the summer, the highest species variation was found in the spring. The collected data contribute considerably to the knowledge of the aphid-transmitted virus epidemiology in economic crops.

02-085

THE SPECIES *ADELPHOPHYLUS* ON THE BALKAN PENINSULA

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The starting point for our research was Wagner's claim that the genus *Adelphophylus* is represented by 1 species - *A. balcanicus* Korm. (Wagner, 1975.). The study of the morphological, ecological and biogeographical characteristics has lead us to the conclusion that there exist 2 different species: *Adelphophylus balcanicus* and *Adelphophylus breviceps*. Firstly, there is the difference in the morphological structure of the genital armature of the males. *A. balcanicus* has "handshaped armature" on vesica, whereas *A. breviceps* does not. Secondly, the populations of *A. balcanicus* inhabit *Verbascum* species only, whereas *A. breviceps* has a wider ecological valency and is found from the zone of deciduous forests to the zone of mountainous habitats (meadows and pastures). Thirdly, on the territory of the former Yugoslavia, in Macedonia, the populations of *A. balcanicus* are concentrated in the Northwest, and the populations of *A. breviceps* in the East. Although their respective ranges are separated, they sometimes overlap, which further points to the claim that there are two different species of the genus *Adelphophylus*.

02-087

VERTICAL DISTRIBUTION OF THE SAWFLIES (HYMENOPTERA: SYMPHYTA) OF VELEBIT AND BIOKOVO MOUNTAINS

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Total of 101 taxa of sawflies have been registered on Mt. Biokovo and 220 on Mt. Velebit, 127 for its southern region and 180 for its northern region. Forty percent of species are present on both regions. Only 23 species are common for Biokovo and Velebit.

Distribution of species are connected to vegetational belts: 6 on Biokovo, 7 on North Velebit, and 9 on South Velebit. The lowest vegetational belt of Biokovo (0 to 300 m) is *Orno-Quercetum ilicis* having 20 species of sawflies. *Quercus-Carpinetum orientalis* is the lowest belt of North Velebit with 35 species, while *Paliuretum adriaticum* of South Velebit has only 11 species. With sawflies richest belts are located on Biokovo and North Velebit between 900 and 1350 m above sea level. The *Pinetum mugii illyricum* belt has only three characteristic sawfly species on South and three on North Velebit.

Families Pamphiliidae, Siricidae, Orussidae, and Cephidae were not registered for Biokovo; while for Velebit we have no data only for the family Xielidae.

02-088

BIODIVERSITY OF NEW ZEALAND BEETLES (INSECTA, COLEOPTERA)

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The beetles are the largest order of organisms, comprising over 350 000 described species worldwide. Approximately 5235 species are described for New Zealand, including 313 introduced. They belong to 82 families in two suborders, Adephaga and Polyphaga. The suborders Archostemata and Myxophaga, although present in Australia, are not represented. Of the 82 New Zealand families, none is endemic, and four contain only introduced species (Archeocrypticidae, Gyrinidae, Lycidae, and Trogidae). Eleven families are represented by more than 100 described species. The numbers of Curculionidae, Staphylinidae, Carabidae, and Scydmaenidae in proportion to the total number of beetle species in New Zealand are similar to the proportions in Australia. However, Colydiidae and Scirtidae are particularly diversified, and are relatively better represented in New Zealand than in Australia. The New Zealand beetle fauna is distinguished by the absence of many major lineages, a high level of endemism, which in many groups is over 90% at the specific level and over 43% at the generic level (e.g., Staphylinidae), and the radiation of many groups of genera and species. The origins of New Zealand's beetle fauna are still poorly understood. They are likely to be varied, including Gondwana elements and elements which arrived here by short and long-distance dispersal recently and in the remote past. Phylogenetic studies within and outside New Zealand, are needed in order to establish the origins of the fauna. The size of the New Zealand beetle fauna is consistent with species number/land area relationships in other areas around the world, including the United Kingdom.

02-090

CURRENT STUDIES ON THE WHITEFLY FAUNA OF THE MEDITERRANEAN BASIN (HOMOPTERA ALEYRODIDAE)

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Since the publication of a catalogue of the world's whitefly, in 1978, there have been several local faunistic reviews of whiteflies within the Mediterranean area, and these have often been accompanied by descriptions of new species. Twelve new species have been described from the area during this time. Work is now progressing on the whitefly fauna of the whole of the Mediterranean Basin, comprising over fifty species, and a revision is under preparation, presenting also a complete identification key. In the present note, general biological, ecological, systematic and distributional data will be presented, including host-plant records and known geographical range. The zoogeography of the group will also be discussed.

02-089

NEW DATA ABOUT THE CARABOIDEA FROM EXTREMADURA (SPAIN)

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The Natural Region of Extremadura is a place that have a good conservation situation, and included some protected areas like the National Park of Monfragüe.

Extremadura has an extension of 41602 Km², and altitudes are between 200 and 2401 m, it is located in the South West of Spain. The preserving situation is special, one of the most important ecosystem is named "Dehesa", and is the result of the rinsing of the old Mediterranean forest, this is a very good example about the equilibrium between the nature preservation and the economic improvement.

The knowledge of the entomological fauna is very poor, particularly about Caraboidea, only exist partial data (Uhagón, 1876; Gayubo et al, 1987), with the help of a project of Spanish government (DGICYT PB88-0377-C02-00 and PB92-0121), a complete study about the Caraboidea of this area has been done.

The attainment of data was made in the period 1990-1991, during all seasons of the year and with different sampling techniques.

In the present communication, we have a preliminary check-list of the Caraboidea from Extremadura, and we do special mention about recently described species like: *Typhlocharis gomezi* Zaballos, 1991, *T. pacensis* Zaballos y Jeanne, 1987; *T. belenae* Zaballos, 1983; *T. jeannei* Zaballos, 1989, *T. carpetanus* Zaballos, 1989; *T. portilloi* Zaballos, 1991; *T. wrasei* Zaballos y Farinós, 1995; *T. hiekei* Zaballos y Farinós, 1995; *Geocharis iborensis* Zaballos, 1990; *Geocharis julianae* Zaballos, 1989 or about changes in the distribution of other, like *Callistus lunatus* Fabricius, 1775; *Acupalpus ibericus* Jaeger, 1988 and others.

02-091

A ZOOGEOGRAPHICAL ANALYSIS OF THE SICILIAN SCALE INSECT FAUNA (HOMOPTERA COCCOIDEA)

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Zoogeographical studies on the Sicilian scale insects fauna have been carried out, whose interest derives from the geographic position of the Island (in the middle of Mediterranean basin) and its paleogeographic and paleoclimatic origin. The approximately 160 species of Homoptera Coccoidea, listed up to now in Sicily, mostly belong to the families Diaspididae (45.91% of the total number), Pseudococcidae (23.27%) and Coccidae (12.58%). Scarcely represented are the families Eriococcidae (5.03%), Asterolecanidae, Kermesidae, Margarodidae (each one 3.14% of total number), Acleridae, Cerococcidae, Cryptococcidae, Micrococcidae, Ortheziidae, Phoenicococcidae (complexively 3.79% of total number). According to the distributional patterns recently elaborated by Italian researchers and currently used in faunistic studies of Western Palaearctic area, these *taxa* should be separated in: cosmopolitan or subcosmopolitan elements (42.14 % of total number), distributional patterns with wide distribution in Palaearctic region (25.18%), distributional patterns with wide distribution in Europe (4.40 %), distributional patterns with wide distribution in Mediterranean basin (19.50%) and elements with restricted distribution area (6.91). The Sicilian fauna, in its complex, appears very rich of Palaearctic and Mediterranean typical elements, while it is poor of African ones. The high percentage of cosmopolitan or subcosmopolitan elements shows how the anthropic activities are one of the most important factors of passive diffusion for Homoptera Coccoidea. Lacking zoogeographic studies on scale insects fauna on close areas, it is very difficult to understand if species with a restricted distribution pattern have to be considered endemic element or more probably they are also present, but unrecorded, in close areas.

02-092

THE BIOGEOGRAPHY OF BUPRESTIDAE (COLEOPTERA) OF THE NON-TROPICAL REGIONS OF THE SOUTHERN HEMISPHERE

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The distribution of selected groups of Buprestidae of the Southern Hemisphere is discussed. A variety of distribution patterns are exhibited by groups in the non-tropical regions of the Southern Hemisphere. Some of these distribution patterns might be interpreted as indicating a gondwanic origin for these groups, but other groups have non-tropical bihemispheric distributions suggesting that other interpretations are possible.

02-094

PRELIMINARY DATA ON THE MACROLEPIDOPTERA OF THE NATURAL RESERVE OF VENDICARI (SIRACUSA, S. E. SICILY) AND FIRST RECORD FOR SICILY OF *LEUCANIA PALESTINAE* STAUDINGER, 1897 (LEPIDOPTERA: NOCTUIDAE)

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First collections of Lepidoptera have been done of the Natural Reserve of Vendicari (Siracusa, S. E. Sicily).
As a whole, 41 species have been identified and among them, the followings 3 were new for the Sicilian fauna: *Cyclophora annulata* (Schulze, 1775) (Geometridae), *Leucania joannisi* Boursin & Rungs, 1952 (Noctuidae), *Leucania palestinae* Stdgr. (Noctuidae). The last one is also new for continental Italy.
Other interesting species collected are the followings: *Chlorissa faustinata* Milliére, 1868, *Scopula emutaria* (Hübner, (1809)), *Casilda consecraria* (Staudinger, 1871), *Platysenta viscosa* (Freyer, 1831), *Discestra sociabilis* (de Graslin, 1850).

02-093

THE COLEOPTERA OF THE MARSH OF FUCECCHIO, TUSCANY (ITALY)

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The Marsh of Fucecchio is one of the largest non-littoral marshes in Europe (1800 hectares). This marsh has an exceptional botanical importance representing a relict of the "tundra" in the middle of the Mediterranean area, with a rich group of boreal and arctic-alpine entities, survived after the last post-glacial period.
Some fragmentary informations were available on the fauna of Coleoptera of this habitat, and only recently a volume on this argument has been published (Bordoni, 1995), reporting the results of a thirty year field study. A total of 999 species of Coleoptera (71 families) have been identified and 851 (29 families) have been considered deeply; of these 236 are helobious, living in ten Coleopteran associations in some ecological zones (*Lemna*, *Phragmites*, *Carex elata*, *Quercus coenosis*). The Fucecchio marsh offers one of the last refuges for a remarkable number of species of great ecological and biogeographical interest, with a prevalently northern distribution. Some species, typical in the Central Europe marshlands, are reported for the first time south of the Appennines. The research reveals that Fucecchio hosts a much richer coleopteran fauna, both in number of species and individuals, that any other such habitat studied in Italy. It also shows that some species extremely rare are abundant in this biotope and points out the presence of an high percentage of European elements s.l. with a prevalently northern distribution and a fair number of entities of Siberian origin. As many as 23% of the helobious species find their southern limit in Tuscany and, in some cases, in the Fucecchio marsh itself; their distribution is obviously dotted since it is restricted to wetlands in the sense of refuge.
These results contributed to underline the great importance of the Fucecchio marsh as an high biodiversity habitat in Italy, that deserves urgent attention and protection.

02-095

BIOGEOGRAPHY AND BIODIVERSITY OF MALAGASY MAYFLIES (EPHEMEROPTERA): THE GENUS *CHEIROGENESIA* (PALINGENIIDAE)

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Although belonging to the Afrotropical Realm, Madagascar occupies an peculiar position since some of its zoological and botanical elements undoubtedly present more affinities with the Oriental Realm. As for other groups, Malagasy mayflies (Ephemeroptera) exhibit a high proportion of endemism. *Cheirogenesia* is an endemic genus and up to now considered as monospecific. Intensive field samples over the last four years let us to discover several new species.
In this work, biogeographical affinities of this genus with other Palingeniidae are quoted and an Oriental origin for this family is proposed.
Factors involved in the distribution of *Cheirogenesia* are presented. As this genus clearly colonizes two types of habitats, ecological and phylogenetic relationships among actually known species are also discussed.

02-096

FAUNAL RECORDS ON COLEOPTERA BYRRHIDAE CAUGHT WITH PITFALL TRAPS IN THE WESTERN JULIAN ALPS (NORTH-EASTERN ITALY)

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The presence of Coleoptera Byrrhidae was monitored on Mount Canin and Mount Montasio (western Julian Alps, north-eastern Italy) by using pitfall traps (Barber traps) from May 1989 to April 1991. The traps contained a saturated solution of sodium chloride in vinegar (7% acetic acid) which was replaced every three weeks. The following environments were investigated: two alpine grasslands (1580m and 2155m above sea level), two thickets of *Pinus mugo* Turra (1595m and 1775m), two willow groves (1220m and 1770m), two beech woods (840m and 1400m), two mixed spruce-beech woods (1010m and 1250m), two ski slope meadows (1170m and 1210m), an arid stony meadow (1010m) and the edges of a dry rill (1170m). In all the sites the rocks were calcareous or dolomitic.

A total of 14 species, out of 42 known in Italy and out of 18 known in the western Julian Alps, were collected: *Simplocaria semistriata* (F.), *S. carpathica* Hampe, *Morychus aeneus* (F.), *Pedilophorus auratus* (Duftschmid), *Cytilus sericeus* (Forster), *Byrrhus fasciatus* (Forster), *B. pilula* (L.), *B. pustulatus* (Forster), *B. luniger* Germar, *B. signatus* Panzer, *B. gigas* F., *Curimus erinaceus* (Duftschmid), *Curimopsis paleata* (Erichson), *C. carniolica* (Ganglbauer). Nine of these species are more or less widely distributed throughout Europe; the remaining are Palearctic or Holarctic. *Byrrhus luniger* is recorded for the first time in eastern Italy and *Curimopsis carniolica*, an endemic species of the eastern Alps and the Illyrian area, occurred for the second time in Italy, about one century after the first finding. *Pedilophorus auratus* was the dominant taxon in the samples and also the most eurytopic species. Most individuals and species were collected from June to July. Pitfall traps can be used in studies on the bryophagous beetle fauna, in the same way as they are used for the ground beetle fauna.

02-098

CHANGES IN CARABID'S FAUNA OF BELARUS' FOR THE LAST 150 YEARS

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In second half of last century at the eastern part of Belarus such common now species as *Carabus nemoralis* Mull. and *Amara majuscula* Chd. were absent. European species *C. nemoralis* the first time was found in vicinity of Vitebsk in 1940, and after 1960 it was the common at all terrain. Approximately at the same time sibiric species *A. majuscula* moved to Belarus from east. For the last 10 years european species *Nebria brevicollis* (F.) migrates from west to the Central Belarus.

The drainage of Polesie's peat-bogs was the possible reason of moving to the nord such stepp species as *Calosoma denticolle* Gebl., *C. investigator* (Ill.), *Calathus halensis* (Schall.), *Poecilus punctulatus* (Schall.), *Amara tricuspidata* Dej., *Harpalus servus* (Duft.), *H. pumilus* Sturm, *H. froelichii* Sturm, *Anisodactylus signatus* (Pz.), *Microlestes maurus* (Sturm).

Known from the past *Callisthenes reticulatus* (F.) and *Lebia cyanocephala* (L.) not found from the beginning of present century.

02-097

BIOGEOGRAPHY OF MIRIDAE OF CATALONIA (SPAIN) (INSECTA: HETEROPTERA)

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In the area of Catalonia (Spain), 370 species of Heteroptera Miridae have been recorded. As a first part of the work, a taxonomic check-list was already published. In the present communication, a biogeographical analysis of this check-list is given in a series of maps. They show how the different biogeographical categories are represented all over the area of study, taking into account the geomorphological usually recognised in it.

02-099

MONTHLY DISTRIBUTION OF ICHNEUMONIDAE (HYMENOPTERA) IN CENTRAL TAMAULIPAS, MEXICO

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Ichneumonids are very important to control some Lepidoptera and other pests. For that reason, it is important to know when are more abundant. With nets, biweekly and diurnally, we collected Ichneumonidae in 12 month period in a locality of Central Tamaulipas, in a Oak forest.

718 specimens in 15 subfamilies from 77 genera were obtained. Cryptinae (216) specimens, Ichneumoninae (137) and Pimplinae (133) were the subfamilies more collected. Only Cryptinae and Ichneumoninae were collected all the year.

None of the genera were obtained all months. *Coelichneumon* and *Setanta* were present in 10 months, *Ischnus*, *Baltazaria*, *Hoplismenus*, *Dusona*, *Eudeleboea* and *Netelia* in 9, and *Pimpla* and *Neotheronia* in 8. However, *Pimpla* (7.2%), *Eudeleboea* (6.1%) and *Enicospilus* (5.5%) were the commoner genera. In March, 21.3% of individuals was collected, followed by October (12%) and December (10.4%) On the other hand, 43 genera (56%) were represented only by 8.4% of individuals, with only 1-3 per genus.

02-100

PIMPLIFORM ICHNEUMONIDAE (HYMENOPTERA) IN THE COLLECTION OF HYMENOPTERA, FACULTY OF AGRONOMY-UAT, MEXICO
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Since 1979, Hymenoptera has been collected in order to form the best collection in northern México, including Ichneumonidae. México is specially rich-species, 535 spp. were recorder by Townes & Townes 30 years ago.

In the State of Tamaulipas, ichneumonids has been collected with nets. This report only includes pimpliforms. Three subfamilies 11 genera and 16 species are recorded. The more abundant genera is Neotheronia (Pimplinae) with 145 specimens (56.64%).

The other species collected are Epirhyssa mexicana Cresson (Rhyssinae), Ganodes sp. and Ganodes n. sp. (Poemeniinae), Acrotaphus sp., Pimpla sanguinipes Cresson, Pimpla punicipes Cresson, Pimpla caerulea Brullé, Pimpla sp., Scambus sp., Calliephialtes sp., Iseropus sp. and Dolichomitus sp. (Pimplinae), which were determined by Dr. C. C. Porter.

November was the month with more specimens followed by March and February. More diversity were found in more humid localities, and in November, too.

02-102

A NEW APPROACH TO THE OBJECTIVE ZOOGEOGRAPHICAL REGIONALIZATION

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An new computer-aided method of the objective zoogeographical subdivision of the territories based on the data of the discrete locality faunas is suggested. The techniques constituting the new method are demonstrated on an artificial example with the known final result. The new method is tested on the data on tenebrionid beetles distribution in the Caucasus and Mongolia. This method enables using the primary data on the records of definite taxa in the discrete localities accumulated in the computer databases.

- The new method is based on the following theses:
1. Cluster analysis, being used for regionalization, is used not for faunas of plots subjectively chosen by an investigator but for objectively registered faunas of discrete localities.
 2. The comparison of faunas of discrete localities is preceded by the detailed computer analysis of available information when it is possible to select the reliable and significant data.
 3. The re-count of matrix of locality similarity and clusters at every step of clusterization is made not on the basis of calculation of their average similarity, but on the basis of uniting the faunas of blended localities or clusters, and new calculation of their similarity.
 4. Cluster analysis accounts not only the similarity of locality faunas, but also physical distance between these localities, calculated from their exact geographical co-ordinates. It allows to smooth accidental similarity of remote localities, which often appears because of unadequate knowledge of their faunas and of accidental coincidence.
 5. The process of cluster analysis of locality faunas will go both in computer memory (as a transformations of similarity matrices), and on display (as a dynamic transformation of differently coloured sectors on the map). It gives the investigator a unique chance to control visually every step of clustering process of localities and to get, when necessary, any additional information on each step.

02-101

BIODIVERSITY AND BIOGEOGRAPHY OF THE ECUADOR MANTIDS (INSECTA MANTODEA)

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The author has compiled a list of 50 species of Mantids present in Ecuador by reviewing the data reported in the literature and adding his personal observations which are still being processed. Analysis of their geographical distribution shows that 38% of the species presents a wide distribution, 42% is present a limited area, comprehending only Perú and Colombia, and that 20% is endemic. The abundance of species and especially the discrete number of endemisms are related to paleoclimatic events that have favoured the formation of new species in this place.

02-103

PRIORITY AREAS ANALYSIS: A THAILAND EXAMPLE USING OWLS (AVES: STRIGIDAE), HAWKMOTHS (LEPIDOPTERA: SPHINGIDAE) AND TIGER BEETLES (COLEOPTERA: CICINDELIDAE)

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Setting priorities in biodiversity conservation requires that explicit, efficient and appropriate methods are developed and made available to conservation managers. The principle of complementarity is fundamental to the most efficient of these methods. Given a goal of a single representation of each taxon, complementarity analysis of data sets of owls, hawkmoths and tiger beetles in Thailand yields near-minimum sets of 6, 14 and 34 areas respectively. The consensus of these sets comprises 48 areas. However, when the data are combined into a single data set, complementarity analysis gives a more efficient solution of 46 areas. Over 90% of the owls, hawkmoths and tiger beetles are already represented as a minimum of one population within the current protected areas network of Thailand. However, an additional 18 areas are still required for complete representation.

02-104

FIRST RECORD FROM ITALY OF *MONARDIA OBSOLETA* EDWARDS
(DIPTERA: CECIDOMYIIDAE LESTREMINAE)

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During the early spring of 1995, a number of mature larvae of *Monardia obsoleta* Edwards have been found on young fruiting bodies of the annual many-coloured polypore *Trametes* (= *Coryolus*) *versicolor* (L.: Fr.) Pilat, growing on decaying broadleaf tree branches fallen to the ground, in different wooded areas of the litoral Tuscany. At the end of April 1995 pupae and adults of the species have been obtained from this material.

The main taxonomic features of *M. obsoleta*, first recorded from Italy, are described and illustrated by drawings, light stereomicroscope photographs and SEM micrographs.

The orange elongated larva has a small weakly sclerotized head and relatively short antennae. The sternal spatula anteriorly bears three sclerotized teeth.

Adults show a typical eye-bridge and 3-segmented palpi. Male presents a wing length of about 1.3 mm, antennae 14-segmented and styles of the hypopygium with a short terminal spine. Female has a wing-length of about 1.8 mm, antennae 17-segmented and two roundish sclerotized spermathecae.

02-106

THE *BOMBINI* OF THE NATIONAL PARK OF SIBILLINI MOUNTAINS (CENTRAL ITALY). (HYMENOPTERA, APIDAE: *BOMBUS* LATR. AND *PSITHYRUS* LEP.)

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The Authors have studied the faunistic composition of bumblebees (*Bombus* Latr.) and their social parasites (*Psithyrus* Lep.) in the National Park of Sibillini Mountains (central Apennines) on the base of nearly 2500 specimen caught in 3 years.

The park spread over about 70000 ha, in the territory of Umbria and Marche regions, within an altitude range of 400 - 2476 meters.

15 species of bumblebees have been found, with 17 subspecies, and 5 species of *Psithyrus*, with 7 subspecies.

One species, *Bombus mucidus* Gerst., already known in central Italy, has been found in this zone for the first time.

Data about the species' corology and the faunistic composition related with the altitudinal vegetation zones, and observations on the flora foraged, are provided.

02-105

MEASURING BIODIVERSITY OF ANTS (HYMENOPTERA: FORMICIDAE)

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The purpose of this study is to show effectiveness of a time unit sampling methods of ants. In measuring biodiversity of ants in a local fauna, in particularly of the tropical regions, a surveyor faces various practical impediments. Among them, time would be one of the major factors affecting quality of a survey. Under such condition, a time unit sampling (TUS) would be an effective way. This is a method collecting as many ant species as possible during 30-min. visual manual search on the surface of the ground, under stones or wood bark, and around the base of tree trunks.

In Okinawa Island (28°N x 126°E), Japan, 41 species belonging to 28 genera of 4 subfamilies were collected by TUS from 12 sampling plots, which corresponds to 60 % of the total number of species or less. The ratio of eurychoric species is higher in open land.

In addition, the results of the surveys in other areas of Japan, Bangladesh, SE Asia, and Panama are presented. The utility and limits of the sampling are also discussed.

02-107

MYCETOPHILOIDS (DIPTERA, MYCETOPHILOIDEA) OF THE "CHERNEVAJA TAIGA" OF THE SOUTH OF WESTERN SIBERIA

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"Chernevaja taiga" is the relict dark coniferous forest (Subnemorosa) with the fir, the fir spruce, the cedar pine and the admixture of the aspen. For the first time 94 species of Mycetophiloids for the middle mountain taiga of the western main slope of Kuznetsk Alatau mountains are described. These species are included in 24 genera of 6 families: *Bolitophilidae* - 7 species (*Bolitophila* - 6, *Messala* - 1); *Ditomiyidae* - 1 (*Symmerus*); *Keroplastidae* - 1 (*Keroplastus*); *Macroceridae* - 2 (*Macrocera*); *Sciophilidae* - 15 (*Mycomyia* - 8, *Polylepta* - 1, *Neurotelia* - 1, *Platinia* - 1, *Leia* - 2, *Monoclona* - 1, *Acnemia* - 1); *Mycetophilidae* - 68 (*Anatella* - 1, *Exechia* - 11, *Rhymosia* - 4, *Brachypeza* - 1, *Allodia* - 5, *Brevicornu* - 1, *Trichonta* - 8, *Phronia* - 7, *Mycetophila* - 26, *Sceptonia* - 1, *Epicypa* - 2, *Delopsis* - 1).

Mycetophiloids of the coniferous middle-mountain forest are the inhabitants of aspen-fir, pine-fir and fir biotopes. They represent both the specific ecological group of the dark coniferous forest and the mountain-taiga faunistic complex. The zoogeographical elements of this complex include the following species: cosmopolitic species - 2 (2%), holarctic - 14 (15%), transpalearctic - 18 (20%), eurosiberian - 16 (18%), conditionally siberian - 43 (45%). The conditionally siberian species are known only on the territory of Western Siberia and their area is studied insufficiently. In addition, 5 new species (*Leia* - 1, *Monoclona* - 1, *Exechia* - 1, *Brachypeza* - 1, *Mycetophila* - 1) are described as the possible paleoendemics - the relicts of the glacial epoch.

02-108

INSECTS IN THE CANOPY OF A TEMPERATE RAINFOREST: THE CARMANAH PROJECT**R.A. RING, N.N. WINCHESTER**Biology Department, University of Victoria,
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The Upper Carmanah Valley on S-W Vancouver Island lies within the Coastal Western Hemlock biogeoclimatic zone and forms a small part of an extensive Coastal, Temperate Rainforest (CTRF) biome on the west coast of British Columbia. A canopy research station has been established in old-growth Sitka spruce and this species has been studied intensively with respect to canopy and forest floor insect/arthropod diversity since 1991. More than 1.2 million insects and other arthropods (mainly spiders and mites) have been collected using a variety of trapping techniques. Results show that perhaps the most interesting habitat contained in the canopy is that provided by the 4-28 cm deep moss mats that are formed on a well developed soil layer on the upper surfaces of large branches. These mats are primarily composed of four moss species which are also abundant on the forest floor. Collembola (springtails) and Acari (mites) dominate this canopy soil/litter habitat, an observation that has not been documented in any previous canopy study. Our preliminary list of mites indicates that significant differences exist at the species level between canopy and forest floor and that several of the canopy species are new to science. The same trend applies to the Collembola. An informative view of canopy arthropods can also be gained by placing them in feeding guilds. The guild structure in our study is dominated by photophagous and predator/parasitoid species. The high incidence of a predatory/parasitoid guild also supports the results of other studies which indicate that herbivory in mature, structurally-complex forests is relatively low.

02-110

SPECIES OF GENUS *EUTRICHOCAMPA* (DIPLURA, CAMPODEIDAE) ON BALKAN PENINSULA**Bela Blesić**Institute of Biology, Faculty of Sciences, University of
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According to data from literature the genus *Eutrichocampa* Silvestri, 1902 is not widely distributed in Europe. However, the fauna of the family *Campodeidae*, and especially of the genus *Eutrichocampa*, is insufficiently investigated in our country. The first date about the occurrence of this genus in Yugoslavia is given by Condé (1947), on the basis of data recorded Condé, he also mentioned presence in Yugoslavia. Condé (1947) discovered *Eutrichocampa* in the West Serbia, in the vicinity of Novi Pazar.

From 13 species known in the world, in Europe 6 species belonging to the genus *Eutrichocampa* have been described up to date. *Eutrichocampa remyi* Condé, 1947 was found on the Balkan peninsula in Serbia (Yugoslavia) and Greece Macedonia. *E. hispanica* Silvestri, 1932 is distributed in Spain. Only on Rodos (insula) is recorded *E. aegea* Silvestri, 1933. *E. helvetica* Wygodzinsky, 1941 is distributed in Switzerland. *E. (Chaocampa) collina* described by Ionescu (1955). Her subspecies is described as *ithacesia* Condé (1984) from Ithaca (Greece). *E. thamagadensis* Condé, 1948 is distributed in Corsica - France. On Balkan up to date are distributed 2 species.

02-109

Bees genus *Hylaeus* Fabricius, 1793 of Azerbaijan (Hymenoptera, Apoidea, Colletidae)

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Bees genus *Hylaeus* Fabricius, 1793 are presented in Azerbaijan by 36 species. European-Caucasian (13), Mediterranean (10) and West-Palearctic (8) species are broadly presented. There are less species presented in Palearctic-only 3, and East-Mediterranean and endemics of Caucasus and the ones with limited natural habitat are presented only by 1 species.

02-111

CHALCIDIDAE (HYMENOPTERA) OF CENTRAL ASIA: BIODIVERSITY AND HOSTS**S.N. Myartseva, V.A. Trjapitzin¹**Institute of Zoology, Academy of Sciences of Turkmenistan, Ashgabat, Turkmenistan -
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The total list of centralasian wasps of the family Chalcididae includes 82 species from 20 genera. Half of these species are distributed in Central Asia only. 14 species from them are discovered in Tadzhikistan only, 8 - in Turkmenistan and 7 - in Iran only. Palearctic distribution have 32 species, 8 species pass the limit of Palearctic: 7 - into Indomalayan zoogeographical province and one comes across Holarctic. Thus, fauna of chalcidid wasps consists of many centralasian endemics and has considerable diversity of species.

As hosts of parasitic wasps were registered 89 species of insects. The generality of wasps are primary and secondary internal parasitoids of insects from orders Lepidoptera, Diptera, Coleoptera, some Hymenoptera and Neuroptera. Most practical significance in region have some representatives of large genus *Brachymeria*.

02-112

STAPHYLINIDAE (COLEOPTERA) FAUNA OF THE CRIMEA AND ITS ORIGIN

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Reconstruction of the historical development of the Crimean fauna based on the geographical distribution of the species from different habitats has been done. In Pleistocene glaciations most of thermophilous Pliocene species including endemics became extinct in the Crimea (modern endemism level - 2%). Few of them survived in the Southern Crimean Coast refuges. The latter were not available for forest species from Russian plain, the steppe barrier constantly existing. In the glaciations boreal species penetrated the Crimea, but most of them disappeared in Holocene (they survived in the Caucasus). Hygrophilous species came to the Crimea at the beginning of each interglaciation, when it was already warm, the Black Sea was fresh and its level was lower than it is now.

02-114

SPECIES DIVERSITY OF BUTTERFLY COMMUNITIES IN A COUNTRYSIDE COPPICE IN OSAKA, JAPAN

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Transect counts of butterflies were made along a 1.3 km path through a countryside coppice dominated by *Quercus serrata*, Japanese cypress plantations, and farmlands with paddy fields in Osaka, central Japan in 1994. A total of 730 individuals belonging to 45 species was observed during 15 counts from April to November. Both species diversity and density for whole transect ($H' = 4.6$ and 37 individuals/km) were as high as those in other coppices in Osaka. The species diversity was the highest in the coppice edge, the next highest in the farmland, and the lowest in the coppice interior and plantation. The density of butterflies was the highest in the farmland, the next highest in the coppice edge and cutover, and the lowest in the coppice interior and plantation. Although numbers of species and individuals were large from May to June in most environments, they became smaller in the height of summer in the coppice edge, cutover, and farmland, where butterflies increased again in October. On the other hand, butterflies were relatively abundant in summer but decreased rapidly in autumn in the coppice interior and plantation. The results suggest that the mosaic of different environment in the countryside coppice is important in maintenance of the diversity and abundance of butterflies.

02-113

BIODIVERSITY OF DOLICHOPODID FLIES (DIPTERA) IN TROPICAL FORESTS OF AFRICA AND INDIAN OCEAN ISLANDS

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The known Afrotropical and Indian Ocean islands fauna of the family Dolichopodidae is relatively small, with about 500 species ("Catalogue of the Diptera of the Afrotropical Region", 1980). But it is quite possible that the real number of species is more than 1000.

The main aim was to determine the biodiversity and taxonomic status of rare or undescribed dolichopodid species from Tropical Africa, Mascarenes and other Indian Ocean islands.

During the last year the work was concentrated on studying the rich collection of 4000 undetermined species from the Natural History Museum (London). Most of the specimens, provided with geographical and ecological labels, were collected from tropical forests in Uganda, Kenya and Mauritius. The collection probably includes from 100 to 200 undescribed dolichopodid species.

About 500 specimens were identified during the first year of research, belonging to 38 identified species from 10 genera. 2 genera, 23 species and females of 2 known species were first described. The most interesting records are as follows:

Argyrochlamis impudicus Lamb - Seychelles, Mauritius, Sri Lanka, Chagos Is.; *Bickelia subparallela* sp.n. - Chagos Archipelago, Seychelles; *Chrysosoma leucopogon* Wiedemann - Tanzania, Kenya (!), Mauritius, Reunion, Rodriguez, Oriental Region, Australia, Oceania. *Mascaromyia libravitertex* Lamb - Seychelles, Mauritius (!), Chagos Archipelago (!).

02-115

ICHNEUMONIDAE (HYMENOPTERA) FROM THE STATE OF TAMAULIPAS, MEXICO

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Thirty years ago, Townes & Townes recorded 171 genera and 535 species of Ichneumonidae from México, including only 4 genera and 4 species from Tamaulipas. In 1988, Ruíz found 243 genera from our country, based in field collections of this important family. This present report includes recent work done in Tamaulipas, in northeastern México.

Since 1981, ichneumonids have been obtained mainly with aerial and sweeping nets and some with Malaise and UV light traps. Diurnal collectings have been done in Oak forests, pine forest, cloud forest and low deciduous forest. Determinations were done by the author, and some by C.C. Porter and J. Luhman. The specimens are deposited in the Collection of Hymenoptera, at this Faculty.

168 genera from 23 subfamilies are known, it means almost the Townes number for all the country. Cryptinae and Ichneumoninae are the best represented, with 37 and 33 species, respectively. *Netelia*, *Enicospilus*, *Eiphosoma*, *Compsocryptus*, *Pimpla*, *Dusona* and *Neotheronia* were the most collected genera. Nine new records were obtained.

02-116

SPECTRAL SEQUENCE OF THE FOODPLANTS OF THE FAMILY LYCAENIDAE AND RELATING FAMILIES OF BUTTERFLIES AND THE APPERARANCE AND THE GENEALOGY OF DIVERSITY OF THE RELATING ANGIOSPERMS.

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The family LYCAENIDAE has 2 large groups of butterflies: Thecline-group and Polyommertine-group. The investigation of the spectrum of foodplants of each group shows that these 2 spectra crosses at a point X near to the family Hamamelidaceae. This group X includes the families of Angiosperms with very primitive sructures such as the families belonging to the orders CELASTRALES, RHAMNALES and SAXIFRAGALES etc., all of which are seemed to be remaining in various stage of evolution, seeing at the present time-point.

It seems that this group X of primitive Angiosperms has a "multistrata structure" according as the "biological time" of the individual Angiosperms constituting this group. Though this group X arose as the foodplants of LYCAENIDAE, it has an intimate relation to the foodplants of the other families of butterflies RIODINIDAE, NYMPHALIDAE etc..

On the other hand, in the family PAPILIONIDAE, there are very old butterfly-species, called the "living fossils", which are distributed along an Equator of old times approximately 650my B.P.. The foodplants of these "living fossils" are extremely limited: the most part of them is Aristolochiaceae and some parts are Crassulaceae and Papaveraceae. Considering upon these facts, the group X is closely related to these families of foodplants of the family PAPILIONIDAE. Except for these "living fossils", because the family PAPILIONIDAE is also connected to the family PIERIDAE, the above group of Angiosperms X is also naturally connected to the spectrum of the foodplants of the family PIERIDAE.

As a consequence of these considerations, we have come to a conclusion that the group of Angiosperms X is a group of Angiosperms which is originated in the principal part of the GONDOWANALAND and diversified to other places according as the law of the evolution and differentiation.

02-118

PATTERNS OF DIVERSITY IN COLLEMBOLA FROM MONTANE WET FORESTS OF HAWAII

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Species diversity and community structure of springtails are compared for mid-elevation rainforests on the islands of Molokai and Hawaii. Elevation, substrate age and the history of feral pig activity all significantly influence Collembolan community composition. Historical surveys of Collembola in Hawaii are reviewed and the utility of using springtails as biological indicators of changes in Hawaiian ecosystems is discussed.

02-117

A PRELIMINARY REPORT ON THE STUDY OF THE BUTTERFLIES IN THE ZHONGTIAO MOUNTAIN

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Zhongtiao Mountain is situated in the southeastern Shanxi Near Mt. Huanggutun, there are about 667 ha primeval forest. It is rich in natural resources. Some scientific surveys on plants and vertebrates had been previously carried out in this region. But, the gap is in the insect fauna. So, an investigation on the butterflies have been conducted. 67 species belonging to 7 families, have been obtained. The fauna is composed of Palaearctic and Oriental elements with Palaearctic elements dominant. The geographical distribution of Palaearctic faunal elements is in the pattern of northern China. The Oriental elements are composed of elements from southwest China and middle China. The intruding channel of the elements may be through two ways: the first is from Qinling mountains, and the second is from niddle China along the junctures of Shanxi, Hubei and Henan Provinces into Zhongtiao mountain. Therefore, this fact can help us infer that the bound of Oricntal region in Shanxi Province is Zhongtiao mountain.

02-119

HAWAIIAN INSECTS AND EQUILIBRIUM ISLAND BIOGEOGRAPHY

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A complete species inventory of the known insect fauna of the Hawaiian Islands has been published (Nishida 1994). We have used it to analyze island biogeographic patterns by regression analyses. Many analyses yielded strikingly significant results lending support to several theories of island biogeography. Extremely significant results were found for regressions of area and elevation against total number of insect species per island. These results probably reflect the higher heterogeneity/diversity of habitats on larger islands, which in turn may provide resistance to disruption and resulting extinction. An analysis of the number of insect genera against island area further supports this ecological explanation, by reducing the effect of speciation on the regression. Analyses of age and number of insect species are confounded by the reduced area and number of habitats present on the older islands. Thus, it is shown that island area is a better predictor of species diversity than is island age. Regression of the number of single-island endemic species as well as the species:genus ratio against island age, area, and elevation suggest that speciation is fostered by habitat diversity and opportunity for speciation as well as by complex topography and the resulting physical isolating mechanisms. These results do not suggest that rates of speciation are necessarily "explosive" for younger islands, but rather that younger islands harbour more products of speciation due to habitat heterogeneity/diversity and relaxed competition.

02-120

DIVERSITY OF NECTAR RESOURCE UTILISATION IN MALAGASY SPHINGID COMMUNITIES (LEPIDOPTERA: SPHINGIDAE)

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Due to the species-dependent differences in proboscis length, sphingid moths are regarded as highly specialised flower visitors of various plant species. However, little is known about the entire spectrum of flowers visited by certain sphingid species.

Hawkmoth communities of Madagascar show larger ranges of proboscis length (maximum 1.1-26.5 cm) than those of other paleotropical regions. To clarify whether flowers are visited by hawkmoths in a more generalistic or selective way, the whole pollenloads of approximately 2000 captured individuals were examined by means of light- and scanning electron microscopy. In addition, morphometrical and phenological data were evaluated, and the abundance of flowers at the study sites was estimated.

The position of the anthers within all flowers examined, allowed adherence of pollen to all species of sphingid moths occurring, regardless to their proboscis length. According to the composition of their individual pollenload most sphingid moth species turned out to be generalist feeders, both on sphingophilous and on non specialised flowers. Sphingid species varied in the number of visited plant species, as well as in flower constancy.

Individuals of long tongued species carried pollen of a larger number of plant species, while those with short proboscis tended to be more selective than expected. This supports our hypothesis stating that an extreme length of the proboscis may not be caused by flower morphology, but rather by feeding while hovering.

Our research was financially supported by the Deutsche Forschungsgemeinschaft (Wa 258/4).

02-121

PRESENT KNOWLEDGE ON THE ITALIAN PSYLLIDS (HOMOPTERA PSYLLOIDEA)

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Taxonomy, biology and ecology of Homoptera Psylloidea have been largely investigated in Italy during the last fifteen years and a wide review on this topic is presently in press, whose data are summarized here. As a whole, 199 species have been totally recorded, thus confirming the Italian one as the richest fauna presently known in Europe. A lower biodiversity appeared in central and southern Italian regions, compared to the northern ones. An almost uniform diffusion all over the national territory has been shown by 21 out of the 199 species, while, on the contrary, a highly restricted or even punctiform distribution has been evidenced by a group of 24 species; 11 species have been exclusively collected in Italy, up to now. As to biological aspects, polyvoltinism showed to be fairly rare among this insect group, since only less than 20 species perform more than two yearly generations; on the contrary, the greatest part of Italian psyllids (about 110 species) are monovoltine. A selective host plant preference has been evidenced: psyllids live in Italy only on 41 (with Leguminosae, Compositae, Salicaceae and Rosaceae being the most preferred ones) among the nearly 141 families and a bit more than 110 out of the nearly 1,090 genera of Angiosperms recorded in this country.

02-122

ARTHROPOD DIVERSITY IN FOUR MOUNTAIN FORESTS OF THE ITALIAN ALPS

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Arthropod diversity was studied in 4 sampling sites in the Provinces of Trento and Bolzano (Italian Alps) within the activities carried out in the ICP Integrated Monitoring programme.

Two sites were deciduous forests at low altitude (550-700 m), and two sites were Norway spruce and cembra pine forests close to the timber line (1720-1820 m).

Inventory of arthropod species was carried out by pitfall traps, light traps and other sampling methods, collecting both soil and plant dwelling taxa. More than 2,000 arthropod species were identified, belonging to Opiliones, Araneae, Collembola, Carabidae, Staphylinidae, Cerambycidae, Elateroidea, Scarabaeoidea, Heteroptera, Lepidoptera, and other small taxa.

Number, composition and relative abundances of species in the assemblages have been analyzed and correlated to environmental parameters and historical processes. Diversity was higher in the low altitude sites. Deciduous forests accounted for 78% of the total species diversity, whereas coniferous forests only 34%. A strong turnover of species occurred between the two types of forests, and significant variations in species composition were recorded also within forest types, with similar variation patterns in deciduous and coniferous stands. Taxa with greater dispersal power showed a lower faunal turnover. The biodiversity patterns reflect the complex biogeographical history and the ecological heterogeneity of the area.

02-123

QUANTIFYING BIODIVERSITY. MULTIPLE USES OF A NEW DATABASE. THE EXAMPLE OF ACARI (ARACHNIDA).

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Biodiversity gives rise to considerable interests. As it covers many different levels, from intra-specific genetic diversity to functional diversity along food chains, or specific diversity within the biosphere, the quantification of so various aspects needs mathematical tools. The size relations indicate that small species are generally more abundant than larger ones. Therefore, mites being certainly the smallest known arthropods, one could predict that they should be more numerous than insects. Such hypothesis demands also an appropriate means of quantification. Furthermore, a quantification of ecological traits of mites allows also to put in concrete form their often written, but not yet computerized, extraordinary ecological diversity.

The present contribution describes a new database and suggests some applications. The data consists of 7 columns of taxa and features: body length, egg length, clutch size, ecology (habitats, parasitism or phoresy) and sexuality (arrhenotoky or thelytoky). The updated file comprises 7892 species (3020 anactinotrichids and 4872 actinotrichids). The 23 anactinotrichid superfamilies accommodate 76 families and 454 genera. Actinotrichida assemble 77 superfamilies, 239 families and 1310 genera.

The most immediate use of the data consists simply in considering only taxonomic items. This is illustrated by a preliminary example of statistical biosystematics. If we consider each taxonomic category as so many samples, it becomes possible to treat mathematically pure systematic data by affecting the number 1 to each item. Then, one can calculate classic statistics and usual tests. The variation coefficient expresses here the homogeneity of the distribution of species within groups.

The database permits diverse kinds of study of ecological diversity, which may be mathematically expressed by a diversity index. This allows rapid comparative analysis between groups.

A third main use of the database concerns body sizes. First, the range, the difference between the highest and lowest measurement, gives an estimation of heterogeneity, of a group. The classical statistics allow to compare different taxa. With its 79 μm , the tiny *Cochlodius minimus* (Tarsonemida) is certainly the smallest known adult Acari. Graphs of sizes indicate an important character: all the histograms present a negatively skewed distributions, the mode being always inferior to the mean. This asymmetry reflects a remarkable evolutive trend towards miniaturization. This tendency stumbles against an inevitable wall, the lowest limit of size and organization for an adult arthropod; this buffer should be situated around a body length of 70 μm .

From the few examples proposed here, one can conceive numerous other interesting studies. As the present database is largely open, future users will certainly find new subjects of researches and theories.

02-124

BIODIVERSITY OF PSEUDOSCORPIONS IN YUGOSLAVIA: THE ORIGIN AND GENESIS OF ENDEMICS AND RELICTS

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The primordial pseudoscorpion stock had colonised the Proto-Balkans (including Serbia and Montenegro) at the beginning of its existence. Subsequently, it gave birth to a number of phyletic series. Such stem forms inhabited the leaf-litter and humus during or even before the Tertiary. Evidently, the ancient epigean fauna was subjected to local destruction due to unfavourable changes of climate, and the lack of migration or possibilities to shelter.

Biogeographically, the Mediterranean and the Pannonian and Ponto-Caspian regions are the two main refuge zones of European pseudoscorpions. The first region was a refuge to a more thermophilous fauna, the second to the Tertiary elements of Central Europe, which migrated southwards due to climatic and other changes. The Dinaric and Carpatho-Balkan Karst are inhabited by the great number of endemic and relict pseudoscorpions of different lineages - from the Gondwanan to the Aegean. These species and genera represent the last vestiges of an old fauna, which found their shelters in different domains of the vast refugial zone of the Balkan Peninsula. Such forms were later subjected to the divergent differentiation in numerous epigean and underground habitats (the "evolutionary explosion" of species in the so-called "Adriatic angle").

02-126

DIVERSIFICATION OF MILLIPEDES IN YUGOSLAVIA: BIOGEOGRAPHIC IMPLICATIONS

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Out of 98 species of Yugoslav diplopods, classified into 41 genera and 18 families, 17 species and 11 subspecies (or 28.57%) are endemic, either to Serbia and/or Montenegro or to both regions. Of these, 14 taxa are troglobites, while others inhabit epigean habitats.

The pattern of the relictual and autochthonous fauna of endemic and relict millipedes in Yugoslavia has been greatly influenced by the process of karstification and paleoclimatic events. These forms are probably the descendants of the ancient epigean hydrophylic and humicolous fauna. With increasing aridity in the past and the formation of different niches underground, these ancient species either evolved as cave inhabitants, or they colonised suitable niches in different epigean shelters.

The origin and development of the rich endemic and residual fauna of both cave and epigean millipedes in Yugoslavia have been affected by the existence of an ancient epigean fauna, by the process of its destruction under unfavourable climatic conditions, and by the subsequent adaptive radiation, enabling the survival of many endangered species. The biogeographical and morphological evidence points to the fact that the Mediterranean area (and the Balkans; including Yugoslavia) represents one of the main refugia of the diplopod fauna.

02-125

TAXONOMIC DIVERSITY OF SPRINGTAILS (COLLEMBOLA, INSECTA) IN YUGOSLAVIA (SERBIA AND MONTENEGRO)

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The fauna of springtails in Yugoslavia is extremely diverse; out of 240 species, classified into 43 genera and 6 families, only 5 species and 4 subspecies are endemic to Serbia and 4 species to Montenegro; a single species is endemic to both regions, while 4 species occur both in Serbia and/or Montenegro and in adjacent Balkan regions. Seven species occur only in caves and potholes.

The geographic distribution and taxonomic features of endemic and relict springtails point to their different origin and age. The diversification of the rich collembolan fauna in Yugoslavia had been probably due to the continuous development of the ancient Mediterranean stock, its origin to be sought in the Dinaric and Carpatho-Balkan regions. Among the main causes which have affected the evolutionary history of Serbian and Montenegrine springtails, one should emphasise the relative constancy of life conditions in different refugia, as well as the isolation in numerous epigean and underground habitats.

The taxonomic diversity of springtails in Yugoslavia is conditioned by the rich primordial fauna (inhabiting the region in the remote past), by the subsequent survival of different taxa in many habitats, as well as by their adaptation and subsequent differentiation in different niches.

02-127

ZOOLOGICAL DIVERSITY IN THE NETHERLANDS, WITH SPECIAL REFERENCE TO ARTHROPODS

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An inquiry amongst zoological specialists resulted in a survey of the Dutch multicellular animals. To date 25,200 species are known, of which 24,400 can be regarded as indigenous. When expected but not yet recorded species are included, the total is almost 35,000 species. The arthropods, in particular insects, account for the largest proportion with 71.5% for insects, 7.6% for Arachnida and 5.1% for Crustacea. Compared with most parts of the world the Dutch fauna is poor, with no endemic species at all. Currently the fauna is changing much faster than can be explained by natural causes alone. Already 600 species are known to have disappeared from The Netherlands. The decline is especially severe for aquatic insects (70% of Plecoptera, 40% of Ephemeroptera disappeared), Hymenoptera Apoidea (30% of the species is threatened or extinct), Lepidoptera and Coleoptera.

On the other hand the number of exotic species is increasing, in particular in the larger rivers, the sea and biotopes which are influenced by man (planted forests, towns, gardens, etc.).

Knowledge of the fauna is disproportionate: there are comparatively many more specialists for vertebrates than for the most speciose groups of arthropods. Also nature conservancy and policy focus on vertebrates and a few conspicuous ('nice') invertebrates as butterflies and dragonflies. This disproportionate attention for a minor part of our biodiversity neglects the key-role of invertebrates, particularly arthropods, in the ecosystem.

02-128

MORPHOADAPTATIONS AS A FACTOR OF TAXONOMIC DIVERSITY

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Biological diversity (BD) is huge manifold problem, from which its concrete aspects can be separated. One of such aspects is taxonomic diversity (TD) reflecting the complicated structure and the hierarchy of the world of living organisms. From the standpoint of a taxonomist studying composition and relationships between groups of animals or plants BD as an objective reality is reflected in TD of these groups and is eventually assessed by the number of species joined in groups of a higher taxonomic rank. Morphoadaptations arising in the evolution and determining biological progress should be regarded among the most important TD factors. Cause and effect relationship between morphoadaptations and TD has been revealed for the Bombyliidae family, one of the largest Diptera taxa. The type of morphofunctional adaptations of adults of bombyliids is to a large extent determined by two processes: transition to obligate nectarophagy and perfection of flight. This is confirmed by a distinct correlation of many morphological structures of adults with length of the proboscis and with character of flight. All representative small genera have mutual correlation of involved morphoadaptations, whereas large genera have a degree of mutual correlation of many functionally important characters. The high level of species diversity therefore is attained in cases where mutual correlation of morphoadaptations provided for biological progress of the groups in the process of its evolution.

02-130

BIODIVERSITY OF AFRICAN TERMITES: AN EVOLUTIONARY PERSPECTIVE

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In contrast to most other groups of organisms, termites show their highest diversity of genera in Africa, not in the Neotropics. This diversity anomaly is explained by the pronounced environmental changes and fragmentation of biota (especially forests) during the Pleistocene. A further key role to explain the anomaly of biodiversity are the specific biological characteristics of termites to survive in small isolated populations even for long periods of time. Thus the Pleistocene promoted allopatric speciation and evolution of new genera.

Two examples will be presented to illustrate the two factors affecting variation of biodiversity in termites: The lower termite *Schedorhinotermes lamanianus* can survive in small and isolated habitat fragments (lowland forest remnants) over an evolutionary relevant time scale. Such isolated populations show morphometrical and genetical differentiations, which may lead to speciation.

For the genus *Macrotermes* our genetic data provide evidence that the roots of the genus date back to the Miocene, with major diversification within genera in the Pleistocene. During the Miocene the extension of arid savannahs expanded considerably in Africa, facilitating the diversification of fungus growing termites.

02-129

'INSECT HERBIVORY AND BIODIVERSITY IN BELIZEAN FOREST'

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Evergreen and semi-deciduous forest in the Maya Mountains of Belize is subject to regular hurricanes as well as having timber extracted over the past 400 years. Despite the secondary nature of the forest timber is still being extracted, and the impact of this on insect and plant (seedling) diversity is discussed.

02-131

A FAUNISTIC SURVEY OF TERMITES IN PROVINCE OF KHORASAN

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Province of Khorasan with about one -fifth of Iran located on northeast, between 31-38 longitudinal. This province has different climate.

During investigation in 1989-1994, 43 sample of different casts has been collected from nest or during nuptial flight. After ecological, ethological, morphological and micrometrical studying, 7 species identified. The confirmation was carried out by Natural Museum of France and UK. The predominant genus is *Anacanthotermes* Jacobson. This genus nearly distributed in all parts of Khorasan, particularly on pasture.

The identification, distribution and ecological aspect are discussed. The identified species are listed as follow:

Family: Hodotermitidae *Anacanthotermes vagans* (Hagen, 1858), *A. turkestanicus* (Jacobson, 1904), *A. ahngerianus* (Jacobson 1904).

Family: Termitidae *Microcerotermes diversus* Silvestri, 1920, *M. turkestanicus* Luppova, 1973, *M. gabrielis* Weidner, 1955, *Amitermes vilis* (Hagen, 1858).

02-132

CORRELATIONS BETWEEN RANGES OF PHYTOPHAGOUS INSECTS AND RANGES OF THEIR HOST-PLANTS

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Ranges of monophagous phytophagous insects occupy usually only a part of ranges of their host-plants. Phytophagous Chloropidae (Diptera) living on grasses and sedges give striking examples of different correlations. Grasses *Phalaroides arundinaceae*, *Dactylis glomerata*, *Deschampsia caespitosa*, *Phragmites australis* have monophagous species of Chloropidae only in the Palaearctic part of their ranges, but not in the Nearctic part. Flies *Chlorops strigulus*, *Dicraeus vagans*, *D. napaeus*, *Calamoncosis glyceriae* are known in the European part of the host-plant ranges, but not in the Caucasian part. Flies *Chlorops gracilis* and *Dicraeus vallis* are known in the European part of the host-plant ranges, but not in the Asian part. Grass *Scolochloa festucaceae* with Holarctic range has monophagous species *Chlorops scolochloae* only in Kazakhstan. Two alternative hypothesis are discussed: migration ability of phytophagous is lower then of host-plants or ecological requirements of monophagous is more narrow then of their host-plants.

02-134

DISTRIBUTION DYNAMICS OF ORTHOPTERA IN THE NETHERLANDS

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The recent distribution of Orthoptera in the Netherlands was studied from 1990 to 1994 by more than 100 amateur naturalists. The former distribution was estimated on the basis of detailed study of collections. All data are stored in detail in a database with about 70,000 records. The present-day and former distribution of the 45 species of crickets and grasshoppers is now known in great detail and is presented in an atlas on maps with 5 kilometer squares. The Orthoptera fauna did not remain unaffected by the dramatic changes of the Dutch landscape. Strong decline or local extinction was noticed for species characteristic of heathlands and sand-dunes, such as *Psophus stridulus*, *Gampsocleis glabra*, *Decticus verrucivorus* and *Gryllus campestris*. On the other hand, species associated with unstable habitats such as roadside verges and wasteland are thriving. Several Orthoptera are actually expanding their range, and species as *Conocephalus discolor*, *Meconema meridionale* and *Phaneroptera falcata* entered our country recently. Other species have expanded their range within The Netherlands, probably due to the favourable wheather conditions (i.e. *Chorthippus mollis*, *C. biguttulus*). The accurate data can now also be used for nature conservation purposes and also formed the basis for the Red Data List of Orthoptera in The Netherlands.

02-133

HIGH INSECT DIVERSITY IN A POOR HABITAT (DRY SHEEP-RUNS IN HUNGARY)

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Pan trapping was performed in two Transdanubian and one Kiskunság NP dry sheep-runs in order to survey aerial plankton as source of the elements of coleopteran and dipteran assemblages (3 hours exposition time at about noon, 30 sample series, 4x4x0.1 m² samples each with nearly 23 000 insects, incl. 18 915 flies). The pan traps seem a proper device to detect insects in the aerial plankton. The number of species is surprisingly high. Altogether ca 500 species of insects were detected (most of the material was identified to species). Among the rare species collected *Homalometopus platycephalus* (Becker) represents a genus new to the Hungarian fauna. Representatives of coprophagous species, autochthonous species of the grassland and also species not developing on pastures were captured: their ratio in Diptera is ca 30 : 20 : 50 %. Although the species in the last group are not members of the pasture assemblages, they must not be neglected: e.g. semi-natural habitats like sheep-runs serve as stepping stones or buffer zones for species developing in natural mosaic habitats.

Only 18 of the 70 coleopteran species, which were collected on sheep droppings were detected in the aerial plankton, while about a half of the 122 dipteran species found on dung were recorded by pan trapping. The seemingly isomorphic habitat proved to be heterogenic for some of the terricolous species, like *Metopina* sp. Some details of the process by which the small coprophagous species colonize the fresh droppings are revealed. This study was supported by the Hungarian Scientific Research Fund (OTKA Nos 3188 and 16892).

02-135

EVOLUTION AND BIOGEOGRAPHY OF HAWAIIAN MIRIDAE (HEMIPTERA)

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Four of the eight subfamilies of Miridae have native taxa in Hawaii. The fauna is dominated by the subfamily Orthotylinae with 11 genera, 10 of which are endemic to Hawaii. The other subfamilies have endemic Hawaiian species in nonendemic, widely distributed genera. The diversity of the Orthotylinae fauna is unusually high in Hawaii, representing 73% of the Miridae genera in Hawaii compared to 18% of the world fauna. The geographic origins of most of the Hawaiian taxa have not yet been determined, but at least two taxa have Indo-Pacific affinities and one taxon has its sister-group in North America. In diverse lineages almost all species are single island endemics. Species in smaller radiations tend to be more widely distributed. The single representative of the subfamily Mirinae is the endemic species, *Hyalocephalus pellucidus*, which occurs on all the main islands. The endemic predatory orthotylinae genus, *Kamehameha*, has a species on the remote island of Nihoa, one species on Kauai, and another occurring on Oahu, Maui Nui and Hawaii. In the speciose genera *Sarona*, *Nesiomiris*, and *Pseudoclerada*, there is evidence for sequential colonization and speciation from older to younger islands. In *Sarona*, most speciation events have occurred within an island and are hypothesized to occur by novel host plant colonization. Host plants have also strongly influenced the evolution and speciation of *Nesiomiris*, with most species restricted to single species of Araliaceae, with a single clade switching to Aquifoliaceae. Speciation has also occurred via allopatry within islands and areas of endemism can be identified, including the Makaleha Mountains on Kauai, and the Kaaawa area on Oahu.

02-136

THE FUNGIFAGOUS THIRPS (THYSANOPTERA, PHLAEOTHIRIPIDAE) NOWEL FOR THE TERRITORY OF FORMER YUGOSLAVIA

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The hypertrophies caused by mistletoes *Viscum album* and *Loranthus europaeus* on various hosts were collected on several sites in Serbia during 1993 and 1994. The collected material contained a large number of insects, including thrips, as was demonstrated by the use of photoelector.

We have identified 4 species of the family Phlaeothripidae. Of these, the following three belonged to the genus *Phlaeothrips*: *P. bispinosus* Priesner, *P. coriaceus* Haliday, and *P. pillichianus* Priesner. The remaining species was *Poecilothrips albopictus* Uzel. All of these species are characteristic inhabitants of the bark and branches of mainly deciduous trees. They even inhabit the holes in the trees made by some Coleoptera and caterpillars. Their nutrition is specific; they feed on the hyphae and spores of mushrooms - they are fungifagous.

The species *Phlaeothrips bispinosus*, *P. pillichianus*, and *Poecilothrips albopictus* have not been found in the countries on the territory of the former Yugoslavia, whereas *Phlaeothrips coriaceus* was previously found in Croatia only.

02-137

INDEX OF THE SAWFLIES (HYMENOPTERA, SYMPHYTA) OF CROATIA

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Up till now 432 species of Sawflies belonging to the 105 genera and 11 families are noted in Croatian fauna. It is 40% of all known European species, considering the fact that Croatia occupy only 1.2% of European continent.

Within 5 regions in Croatia only 22 species are found in all of them. In East Croatia 255 species (or 59%) are registered, among which 53 are characteristic just for this region. In North Coast region 208 species (48.1%) are registered, with 27 only for this region. The species *Hennedya annulitarsia* which is known only from Gibraltar and Morocco is also found here.

The Highlands and South Coast represented transitional systems from highlands to seashore with equal number of species which is 181 or 182 (or 42%). However, only 25 species are characterized for the Highlands and only 23 for South Coast. Central Croatia is still poorly explored, with 96 species among which 6 are particular for the region.

The peculiarity of species depends on climatic conditions as well as the phytocenosis, characteristic for each of the mentioned regions. (Continental-middleeuropean elements, mountain elements, submediterranean elements and mediterranean elements.)

02-138

POPULATION STRUCTURE, GENETIC VARIATION AND CONSERVATION OF THE CHALK-HILL BLUE BUTTERFLY, *Polyommatus coridon* (Lepidoptera: Lycaenidae), IN SARDINIA

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The *Polyommatus coridon* species complex is present throughout Europe and is characterized by a patchy distribution in colonies localized in suitable habitats on chalk and limestone. In 1987, its presence was ascertained in the central massif of Gennargentu in Sardinia and it was described as a new subspecies, *P. coridon gennargenti*, based on distinctive wing pattern. We analyzed this taxon by polyacrylamide gel electrophoresis of enzymatic proteins and found it genetically well differentiated and isolated from mainland populations of *P. coridon* and related taxa. Absence of gene flow was indicated by the presence of alternative fixed alleles at the *Aat*, *Gpi*, and *Pgm* loci and significant differences of allele frequencies at other loci. The extremely low level of heterozygosity found in the sample examined and the discovery of other small and isolated subpopulations in the same mountain area, led us to further investigated the genetic structure of this taxon in order to asses the degree of genetic variation within subpopulations, and genetic differentiation and gene flow among them. These data and other biological parameters were evaluated with regard to a possible risk of extinction of the Sardinian taxon following environmental changes.

02-139

ANALYSIS OF LITTER ENTOMOFAUNA IN THREE DISTINCT HABITATS IN TROPICAL REGION IN SERRA NEGRA, SP, BRAZIL.

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Litter entomofauna of forest, *Eucalyptus* reforestation area and abandoned pasture land in the tropical environment were compared. Total twenty samples of soil and litter were collected from each site. Soil humidity, pH, granulation and organic matter contents were analyzed. Litter samples, 0,1m², were collected and examined for the composition of entomofauna. Results were compared through Simpson's and Sorensen's indexes, species constancy and richness.

In species richness forest habitat presented 68.2%, pasture 59.9% and *Eucalyptus* 57.7% of the total estimated species. Maximum species diversity encountered were 98.3% pasture, 95.9% forest and 91.7% in *Eucalyptus*. The most common species in forest were Formicidae (sp3 and sp8), Staphylinidae sp1, Coccidae sp1, Gelastocoridae sp1 and Entombyridae sp2 while in *Eucalyptus* and pasture they were respectively Psychodidae sp1 and Formicidae sp 31. Similarity indexes for the three habitats were 47.2% for pasture/*Eucalyptus*, 45.6% for forest/pasture and 43.3% for forest/*Eucalyptus*. Results indicate that species richness was most in stable environment ("forest") while species diversity was highest in unstable transient environment (pasture habitat).

02-140

RICHNESS AND DIET OF LEPIDOPTERAN FAUNA OF THREE HOST PLANT GENERA IN THE CERRADO OF CENTRAL BRAZIL
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The cerrado is a natural vegetation with variable physiognomies and extreme floristic heterogeneity, covering 22% of the land surface of Brazil. The cerrado has an estimated lepidopteran fauna ranging from 6,000 to 10,000 species. This field study was carried out between 1991 to 1995, in four 1 ha cerrado *sensu stricto* areas near Brasília. Fifteen individuals of each of nine species of host plant were censused every week. The three genera of host plants gleaned for caterpillars were: *Byrsonima* (Malpighiaceae), *Erythroxylum* (Erythroxylaceae) and *Qualea* (Vochysiaceae). Caterpillars were collected and reared to adults using leaves of the species on which they were found as food. More than 16,000 host plants were surveyed and we successfully reared adults of 135 species of Lepidoptera. For each plant genus we found around 55 lepidopteran species, with low faunal similarity among host plant genus. Thirty-six per cent of the identified species were polyphagous. Some were extreme generalists such as *Fregela semiluna* (Arctiidae), *Megalopyge albicollis* (Megalopygidae), *Inga haemataula* (Oecophoridae) and *Hylesia schuessleri* (Saturniidae). The majority of the species had very low frequency, and more data are required to provide adequate information on specialized feeders. However, the results obtained indicated that some species exploit only one host plant genus and showed preference for some species within the genus. This was the case with: *Compsiolechia* sp (Gelechiidae) and *Pococera* sp (Pyralidae) on *Qualea*, *Dichomeris* spp (Gelechiidae) on *Erythroxylum* and, *Chiomara punctum* (Hesperiidae), *Cerconota achatina*, *Gonioterma exquisita*, *G. indecora* (Oecophoridae) on *Byrsonima*. The low proportion of host plants with caterpillars (11%) and the high incidence of rare species appears to be a general pattern for the cerrado. The results lead us to conclude that there is a need for more extensive and independent data sets before generalizations about richness and diet of herbivorous can be made for the cerrado.

02-142

EFFECTS OF DISTURBANCE AND FRAGMENTATION ON THE STRUCTURE, DIVERSITY, AND DYNAMICS OF AMAZONIAN BUTTERFLY COMMUNITIES

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The Biological Dynamics of Forest Fragments Project, working in low-productivity upland rainforests 70 km north of Manaus, has given opportunities to follow butterfly communities along the process of disturbance, isolation, and peripheral regrowth of 25 reserves of 1, 10, 100 and 1000 ha. Standardized daily lists (161) of all Papilionoidea observed along transects and at baits were taken between 1980-1995, totalling 35-90 person-hours in each reserve. Of the total 455 species recorded, 88 were seen in only one reserve, while 133 were found in more than half the reserves. During early isolation stages of 12 reserves in 1980-1986, about 32,000 individuals of the common *Solanum*-feeding edge ithomiine (*Hypothyris euclea*) were seen, but it was rare in 1991-1995. The community was dominated by Lycaenidae (255 species) and species of forest understorey sun-flecks (205); 75 species were recorded only on new forest edges. A typical daily list (10 hours) in continuous forest included only 50-70 species; presence of larger clearings or edges increased this by 50-100%. A 10x increase in area of a reserve added only 25% to its total species list, mostly Riodininae; other groups, like larger light-loving species and understorey Satyrinae and Ithomiinae, did not show significant area effects. The turnover (reciprocal of the similarity index) between successive lists in the same reserve averaged 1.63 in 2-4 days, 1.97 after 2-24 months, 2.11 in five years, and 2.40 after change in isolation status, and was larger in continuous forest (2.01) than in isolated reserves (1.76). Most effects on the reserve and daily lists were clearly correlated with disturbance and environmental heterogeneity, rather than size or isolation per se. The general picture of butterflies moving out over the landscape and colonizing any new resources they find, agrees with other diversity studies in richer localities in the SW Amazon. Thus, some butterfly groups can be rapid and sensitive indicators of system disturbance and health. Disturbance-driven fluidity and diversification of butterfly communities in tropical forest has important implications for their monitoring, conservation and management.

02-141

BIOGEOGRAPHICAL PATTERNS OF ALTITUDINAL DISTRIBUTION IN THE SOCIAL WASP FAUNA (VESPIDAE, HYMENOPTERA) OF YUGOSLAVIA

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The analysis of biogeographical patterns of the social wasp fauna was based on the abundant material from the whole territory of the former Yugoslavia (collected within more than 100 years), and thorough field studies conducted in several characteristic areas. It comprises about 15000 specimens from approximately 350 single or composite localities (covering diverse regions and altitudes), with more than 3100 unit-samples (= unique species/locality/date combinations). The altitudinal distribution spectrum of the component species represents the most notable biogeographical pattern of the regional fauna, as a local reflection of both their different area types and broader ecological (bioclimatic) gradients of the region.
The fauna of Yugoslavia (and Balkan peninsula) consists of 4 genera and 21 species of social wasps (*Vespa* - 2 spp., *Vespula* - 4 spp., *Dolichovespula* - 6 spp. and *Polistes* - 9 spp.), comprising a wide range of faunal elements and zonal types, from Palearctic-subtropical *V. orientalis*, to boreo-montane *Dolichovespula* species. The recorded altitudes ranged from the sea level to about 2300 m, but only few exact records were from the zone above 2100 m. Four major, albeit loosely defined altitudinal zones could be recognized (0 m ⇨ 600 m ⇨ 1100 m ⇨ 1800 m ⇨ above), characterized by different subsets of the recorded species and their relative frequencies. The quantitative analysis of the altitudinal distribution of records within and between the zones, in correlation with the habitat preferences of species, enabled a precise recognition of even subtle ecological and biogeographical differences between species, resulting in a hierarchical classification of local faunal elements.

02-143

BANANA BAIT TRAPPED NYMPHALIDAE (LEPIDOPTERA) AS INDICATORS OF HABITAT QUALITY IN A TROPICAL RAIN FOREST (EASTERN IVORY COAST)

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The "Forêt classée de la Bossematié" (ca. 20 South of Abengourou, Eastern Ivory Coast) is the object of an important project from the GTZ (Gesellschaft für Technische Zusammenarbeit, German ODA). Before the beginning of the project, when the Bossematié forest was not yet protected, logging and poaching were very frequent. The forest became consequently a mosaic of different patches. Some of these are large, others small, some are probably still patches of primary forest, others are so deteriorated that they became overgrown by the bush *Chromolaena odorata*, now a major pest of the forest. A reforestation program is now ongoing in the clearings and the old cocoa plantations. Although the Bossematié forest is in a "bad state", from a certain point of view this state can be considered as an asset for specific research. In this forest, all gradations from nearly primary forest to clearings and plantations are present, and this from small patches to large ones. This became the basis for investigating to which extent large, medium to small patches of the forest can contain a fauna of primary forest. The preliminary study was carried out on butterflies and birds (Waltert, i.l.).
The butterfly data are derived from samples of banana baited traps. Possibly this sampling method needs some standardizing, and it is difficult to comprehend what it can really measure, but it is quite efficient: in the Bossematié forest, in a few weeks, 80 different Nymphalidae species were caught (one of which new to science), most of which can be identified and counted in the field. The fact that this sampling method "attracts" the specimens is one of the elements that forces one to be cautious in drawing conclusions.
Among the first conclusions, which could easily be anticipated, is that the species belonging to the Charaxinae, although typical "canopy species", were found quite commonly in traps placed in degraded habitats. These species are good flyers and if degraded patches are situated near more primary ones, the results can easily be explained. But for some typical forest understorey species belonging to the genera *Euphaedra*, *Bebearia* and *Euriphene*, the results were impressive. These seem not to move at all between the forest patches (were rarely found in traps placed outside the patches), most of the species were indicators of forest patches of "good quality" and a single one could only be found in a patch considered of the "best quality".

02-144

DIVERSITY AND SPECIES ABUNDANCE OF SUBTROPICAL FOREST INSECTS(COLEOPTERA:DIPTERA:HEMIPTERA: HYMENOPTERA:LEPIDOPTERA:ORTHOPTERA) IN MACAU, SOUTH EAST ASIA.

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Despite a tiny land acreage comprizing Macau (16.92 sq.Km.),its species diversity is believed to be greater than temperate land areas of comparable size to the north but less than in more tropical areas to the south such as in Malaysia.

A large number of plant species(trees,climbing shrubs,herbs,vines,1296 sp)provide both habitat and a food source for over 145 species of moths and 68 species of butterflies. Rugged hillsides characterize the centers of both islands as well as the Guia hill on the peninsular area connect- ed to China and contain poor soils typicalto that of forested areas in the tropics.

Cerambycid beetle larvae(16sp.)that reside in woody vegetation as well as leaf-feeding adult scarab beetles(10 sp.)are the most abundant among the Coleoptera while the Acridoid grasshoppers (24 sp.) far outnumber species in the family Tettigoniidae(5) or the Gryllidae(7) among the Orthoptera. With the exception of the ants in the order Hymenoptera most of the bees and wasps in the territory are found in the vespoidea(8) and scoliid groups(5) while the pentatomid bugs(16sp) constitute the largest group in the order Hemip- tera. Within the Diptera the mosquitoes(35 sp.) comprize the largest group of fly species due to ample water holding containers. Populations of soil acari and muscoid diptera are limited by the lack of decaying organic matter on the forest floor.

02-146

VARIETY OF THE WING PATTERN IN POPULATIONS OF SOME BLUE BUTTERFLIES (LEPIDOPTERA, LYCAENIDAE) OF THE CRIMEA SOUTHERN SHORE.

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Many of blue butterflies have an expressive wing pattern variability on the ventral wing surfaces that has connected with landscape condition. Differences of the wing pattern variety were researched in populations of *Plebejus pylaon* (F.W.), *Glaucopsyche alexis* (Poda), *Cupido osiris* (Meig.), *Pseudophilotes vicrama* (Mr.), *Plebicula thersites* (Cant.) and *Polyommatus icarus* (Rott.) at the Karadag reservation and arounded territories on May-July 1990.

For comparative morphological analysis next wing pattern characters have taken: 1) colour of the hindwing basal portion; 2) colour of the central portion of the borth wings; 3) expression of the submarginal ereescents of the borth wings; 4) expression of the discal spots and external elements; 5) quantity of the marginal spots and its position; 6) quantity of the basal spots and its position; 7) expression of the white dab(s) between the hindwing marginal and external elements.

Results of investigations have cited in Table 1. Enough high thresholds of the wing pattern variety in populations of *G.alexis*, *C.osiris* and *P.icar*us are supported by wealth of inhabitats of Karadag reservation landscapes and great ecological plasticity of these species. *P.py*laon and *P.vic*rama are the most wouDED and its populations endure damage when landscape destructions taken place in territories arounded the Karadag reservation.

Table 1
Thresholds (i) of the wing pattern variety in lycaenid populations of the Crimea Southern Shore.

Species	Karadag reservation	Arounded (unreserved) territories
<i>Plebejus pylaon</i>	0,5	--
<i>Glaucopsyche alexis</i>	0,8	0,2
<i>Cupido osiris</i>	0,9	0,4
<i>Pseudophilotes vicrama</i>	0,4	--
<i>Plebicula thersites</i>	0,6	0,4
<i>Polyommatus icarus</i>	0,8	0,5

Comparison of observed results have suggested that the wing pattern variety is more high in the Karadag reservation than around it { (0,4 ≤ i ≤0,9); n=875; $\chi^2=36,8$; P=0,5}.

02-145

Xerophilous tenebrionid beetles (Coleoptera: tenebrionidae) from South-East Transbaicalia

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It is well-known that Siberia is not reach of the Tenebrionidae species Nevertheless, this very peculiar group of Coleoptera attraets attention of the researchers in this region The investigated region is situated close to Mongolia, which have quite an authentic and diverse fauna of the Tenebrionidae We registered 15 species of this family in the South-East Transbaicalia (the zonal steppes of the Onon Dahuria and the Uldzah-Thorei valley near large salt Thorei Lakes) Most of them, as expected, are representatives of the Mongolian fauna This fauna have the north-east border in the studied region The table of species is given below

Tribe	Genus	Species
Tentyrini	Anatolica Eschscholtz, 1831	A undulata (Gebler, 1832)
		A aucta (Faldermann, 1835)
	Seytosoma Reitter, 1895	S pygmaeum (Gebler, 1832)
		M angulosa (Gebler, 1832)
Blaptini	Blaps Fabricius, 1775	B rugosa Gebler, 1825, B miliaria Fischer-Waldheim, 1844, B reflexa Gebler, 1832, B variolosum Faldermann, 1835
Platysechini	Platysechis Latreille, 1818	P brevis Baudi, 1876
Opatrini	Gonocephalum Solier, 1834	G. reticulatum Motschulsky, 1853
	Opatrum Fabricius, 1775	O subaratum Faldermann, 1835
	Melanesthes Dejean, 1834	M faldernanni Mulsant et Rey, 1859
Phalerini	Paranemua Heyden, 1892	P bicolor Reitter, 1895
Cryptini	Crypteus Latreille, 1817	C quisquillus Linnaeus, 1761
		C rufipes Gebler, 1830
		B steppensis Kaszab, 1964
Belonini	Belopus Gebien, 1911	

Section 3
Morphology and
Ultrastructure

03-002

HOW DO NERVE CELLS COMPUTE?
DENDRITIC PROCESSING OF MOTION-INFORMATION IN
VISUAL INTERNEURONS OF THE FLY

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Dendrites are not just electrically passive structures acting as simple summators of their synaptic input. Instead, dendrites are, quite in general, equipped with a variety of different voltage-gated ion channels. We are interested in the functional consequences of this complex physiology of dendrites for the computational properties of nerve cells. We investigate this problem in the so-called tangential cells of the fly visual system. There exist about 60 of them in each hemisphere of the fly brain each of which can be identified individually. On their large dendrites, they receive input from hundreds of retinotopically organized local motion-sensitive cells. Due to their location right underneath the surface of the brain together with their two-dimensional arborization pattern, these cells are amenable to a variety of electrophysiological and optical recording methods, and, at the same time, allow for a stimulation of their dendrites by the natural synaptic input in vivo. We combine our experiments with computer simulations taking into account the detailed anatomy of the cells as obtained from 3D-reconstruction. Comparing the response properties of different types of tangential cells, only one of which is equipped with a fast sodium inward current in its axon as well as in its dendrite, we found that cells with fast activating sodium currents can respond to temporal changes in their synaptic input signals up to much higher frequencies than those which lack such currents. Thus, fast sodium currents lead to a frequency-dependent amplification of synaptic signals, enhancing cellular responses specifically to transient inputs which otherwise would be attenuated due to the passive properties of the dendritic tree.

03-001

SPATIAL VISION AND EYE DESIGN IN INSECTS

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Light availability has driven the evolution of the two major compound eye designs found in insects. Superposition eyes, which sacrifice spatial resolution for an improved photon catch, are common in insects active in dim light. Insects active in bright light typically have apposition eyes. These eyes are adapted to provide the maximum resolution possible, often leading to "foveal" eye regions of higher acuity.

Even though superposition eyes are typical for dim-light insects and apposition eyes typical for bright-light insects, there are many fascinating exceptions to this "rule". Many insects have been forced (by competition for example) to occupy niches with light intensities that are not optimal for their eye designs. This has led to surprising optical and neural adaptations which have re-optimised the eye to the new niche. After reviewing the way in which compound eye design affects spatial vision in insects active at different light intensities, I will describe two remarkable eyes which provide exceptions to the "rule": the superposition eye of the fast-flying day-active hawk moth *Macroglossum*, and the open-rhabdom apposition eye of the cave-dwelling beetle *Zophobas*. These two eyes exemplify the ways in which the trade-off between spatial resolution and the sensitivity to light can be radically manipulated during evolution, despite its initial setting by the ancestral eye design.

03-003

THE REGULATION OF DAILY PLASTIC CHANGES OCCURRING
IN CELLS OF THE FLY'S OPTIC LOBE

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The nervous systems of insects exhibit a number of forms of plasticity. Not only are external factors, such as the insect's experience in adult life, important, but intrinsic rhythms also modulate structural and functional properties of the nervous system. In the fly's visual system, for example, two clear cases of structural plasticity have a daily rhythm. In the first optic neuropile, the lamina, of *Musca domestica*, monopolar cells L1 & L2 receive input from photoreceptors R1-R6; L2 in turn feeds back upon R1-R6. The numbers of feedback synapses show cyclical changes. They are more numerous: a) during the night than during the day; and, when the fly is held under constant conditions, b) during the fly's subjective night than during subjective day, thus showing a circadian rhythm. The axons of L1 and, even more, L2 also swell in size during the day, shrinking by night. This second rhythm is apparently controlled by diffuse arborisations from at least two sets of modulatory neurones. One of these, immunoreactive to the peptide PDH, in *Drosophila* also expresses the product of the clock gene *period* (*per*), which is essential for the expression of circadian rhythmicity. The second, LBO5HT, is immunoreactive to 5-HT, which is widely invoked in insect circadian rhythms. Injections of these two putative modulators, or other manipulations of their action or of their cells of origin, are all compatible with a role for both in mediating changes in cell size. They do not distinguish between the actions of the two systems, however, which both increase size in L1 & L2. The LBO5HT and PDH cells both distribute varicose neurites to the lamina, but even though the varicosities are sites of presumed release they apparently lack presynaptic ribbons and direct postsynaptic elements, and thus probably release non-synaptically. Neither the relationship between the size changes in L1 & L2 and synaptic changes in L2, nor the function of either change, is currently clear, nor is the mechanism by which either modulator effects such changes. But the widespread tangential distribution of LBO5HT and PDH neurites throughout the optic neuropiles does suggest that these are merely one of various examples of such plasticity occurring in the optic lobe, that have a recurrent daily basis as consistent as is the coming of each day. Supported by HHMI grant 75195-543701 and MRC grant MT-13242.

03-004

BINOCULAR VISION IN THE PRAYING MANTIS

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Visual guidance of prey capture in mantids involves binocular information, but how the brain unifies the inputs from the two eyes is not known. In behavioural experiments, this problem was analysed by presenting the insect with two or more targets simultaneously. From the results it can be concluded that the visual system is able to match pairs of retinal images which correspond to the same object in space. The matching process requires, however, the targets to be separated by at least 20°. If the gap between the targets is smaller, then each eye fuses the retinal images and the binocular interactions are performed as if a single object were present in the field of view. Binocular target separation is therefore based on a very rough angular scale. One implication of this finding is that the ambiguity problem of stereo-matching does not exist in mantids, because potential false matches are associated with disparities which are too large to be detected by the visual system.

03-006

HOW GRASSHOPPERS SEE AND REACT TO MOVEMENTS.

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In Acridids, the largest neurons which descend the nerve cord from integrative sensory centres in the brain to motor centres of the thoracic ganglia convey information about visually detected movements. These neurones include a group of three which, by responding to input from the ocelli as well as the compound eyes, monitor shifts in the visual horizon and act to stabilise flight attitude. Another large descending neurone responds to widefield rotational movements of the type which elicit optomotor responses, and the presynaptic neurone to this, in the lobula, has been characterised.

The most intensively studied neuron is the descending contralateral movement detector (DCMD), and this is directionally sensitive to movements in depth, responding with accelerating bursts of spikes to objects approaching the eye. The selectivity of the DCMD for approaching rather than receding objects is achieved monocularly through two different visual cues: an increase in the amount of edge in the image; and an increase in the rate of travel of image edges. Each spike in the DCMD is mediated by a spike in the lobula giant movement detector (LGMD). We have discovered that other large neurons in the lobula also display sensitivity to motion of objects in depth. Modelling studies have demonstrated that this selectivity is probably implemented by a simple network connectivity strategy.

03-005

VISUAL DISTANCE DISCRIMINATION IN INSECTS: AN INDEX OF THE USE OF MOTION PARALLAX

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Experiments have shown clearly that some insects use their own movements in visual measurement of the distance to stationary target objects. In doing so, they take advantage of the fact that nearer objects shift at a greater angle and faster than more distant ones (motion parallax). In addition, in the course of evolution, locusts* and mantids have developed the ability to use side-to-side head movements, or so-called peering movements, to make absolute determination of the jump distance to target objects. Although these two species have very different life styles, as expressed in their different morphology as well as in the optical characteristics of their compound eyes, they use quite similar strategies. Locusts enlarge their peering movements (amplitude and speed) with object distance to an extent that keeps retinal image movement about the same at all distances. Mantids also increase peering amplitude with object distance, but keep the peering speed generally constant, whereby it must be assumed that the peering movement (amplitude) is controlled by the image movement (speed). For both species, peering behaviour suggests that the animal's own active movement is taken into account in distance measurement using motion parallax. It is significant that both species require two completely intact compound eyes for absolute distance measurement. In mantids, the area of higher spatial resolution in the most frontal part of the compound eye, i.e. the fovea, seems to play an important role; this has not been studied in locusts. In this context, it cannot yet be said whether parallax measurement is based on a positional or frequency mechanism. During a peering movement, the image of the object could be registered in different areas of the eye (relationship to fovea), or, however, the information on parallax could be contained in the time that elapses between the activation of the sensory cells of neighboring ommatidia or rows of ommatidia. *Collett, T. S. (1978), *J. exp. Biol.* 76, 237; Sobel, E. C. (1990), *J. Comp. Physiol.* 167, 579. Supported by Grant No. P10861BIO from Austrian Science Foundation

03-007

NEURAL MECHANISMS OF POLARIZED SKYLIGHT NAVIGATION IN INSECTS

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Apart from the sun, the e-vector pattern of polarized skylight offers a reference for visual compass orientation. Many insects are able to exploit this polarization pattern for navigation or course control. Although all microvillar photoreceptors are potentially polarization sensitive, it is just a small group of specialized receptor cells in the dorsal rim area of the compound eye that are dedicated for polarization vision. The microvilli of these receptors are strictly aligned along the rhabdom rendering them strongly polarization sensitive. In the rest of the eye the microvilli are misaligned, which reduces or even abolishes polarization sensitivity. Each dorsal rim ommatidium contains two sets of receptors with their microvilli oriented 90° to each other, i.e. they are tuned to different, orthogonally oriented e-vectors. Polarization vision is monochromatic; it is based on UV-receptors (bees, ants, flies), blue-receptors (locusts, crickets) or green-receptors (cockchafer). In several insects the dorsal rim ommatidia are also optically specialized having strongly extended visual fields.

In the optic lobe of crickets, polarization-sensitive interneurons were found. The spike activity of these neurons is a sinusoidal function of e-vector orientation with an excitatory and an inhibitory part, and with the maxima and minima separated by 90°. Thus, these so-called POL-neurons are polarization-opponent neurons receiving antagonistic input from two analyzer channels with orthogonal tuning orientations. We believe that the two channels are represented by the two sets of receptors with orthogonally arranged microvilli present in each ommatidium. The antagonism makes the polarization signal insensitive to intensity modulations and enhances e-vector contrast. Evidence that this antagonism is a general principle in polarization vision is given by behavioural experiments with bees, and by the conspicuous orthogonal microvillar arrangement in the dorsal rim ommatidia of insects belonging to different orders (Odonata, Orthoptera, Coleoptera, Hymenoptera, Diptera). Another, possibly general mechanism is the integration of e-vector information from a large area of the sky. Field measurements using an opto-electronic model of a POL-neuron indicate that this improves signal quality by evening out disturbances of the polarization pattern by clouds or terrestrial objects.

03-008

CENTRAL MECHANISMS OF FORM PERCEPTION IN INSECTS

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The lobula complex of the insect optic lobes has a columnar organisation and layered-terminal structure that is reminiscent of the mammalian visual cortex. Recent recordings from this neuropil described units with 'high-order' properties such as small target selectivity (the 'hypercomplex' property) and orientation selectivity - also reminiscent of cells in the cortex (O'Carroll (1993), Nature 362, 541-543). Given the role of the cortex in processing complex spatial information, it seems likely that these 'cortex-like' pathways are involved in similar tasks. Behavioural analyses provide evidence for relatively sophisticated pattern recognition capabilities in some insects.

I have used intracellular recordings from small and medium-field units in the lobula complex of flies and dragonflies to investigate how the response properties may derive from the anatomical and spatial organisation of the inputs. This reveals a diversity of spatially complex receptive fields. Most cells lack a concentric or circular receptive field organisation and rather have at least two (and in some cases as many as four) spatially distinct sub-regions. These are often elongated and with differing orientation of the long axis. Some sub-regions are inhibitory, while others are excitatory. Dye injections of medium-field units (receptive fields ca. 20° across) reveal an anatomical structure consistent with the spatial properties. In many cases, units have two or more spatially distinct, 'flattened' dendritic regions.

I have yet to encounter units which are exact analogues of specific cell types in the mammalian cortex, but the receptive field organisation of these cells is consistent in many respects with simple 'cortical' models for properties such as orientation selectivity and hypercomplex behaviour. It seems likely that these cells contribute to pathways that provide the basis for tasks such as object and pattern recognition.

03-010

GLIAL JUNCTIONS, DIFFUSION BARRIERS AND ELECTRICAL COMPARTMENTS IN THE INSECT NERVOUS SYSTEM.

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Insects possess a potent blood-brain barrier (BBB) that prevents test tracers injected into the haemolymph (dyes, lanthanum, peroxidase) from penetrating into the central nervous system (CNS). In a few of the most ancient insect Orders, dyes can penetrate from the blood into the extracellular space around the photoreceptors, but recent groups have evolved a blood-eye barrier (BEB) proximal to the retina. This seals or slows the diffusional route into and out of the retina. In muscomorph Diptera, the cells at the BEB interface resemble those that form the general BBB, so that the BEB may simply represent an inward ingression and closure of the older BBB, underneath the retina, cutting this off from the rest of the optic lobe of the brain.

The sealing mechanisms responsible for forming these barriers are still unclear, but are probably associated with a sub-type of septate junction on membranes of particular sheath-like glial cells, which result in very large dimensional changes in the extracellular route. No definitive tight junctions have been shown to exist at the site of either the BEB or BBB, although in the eye these are clearly present just below this, in the lamina neuropil. In the lamina, tight junctions are present on different (epithelial) glial cells, and serve to electrically isolate the extracellular compartment of each synaptic complex (optic cartridge) from that of its neighbours, at a location actually inside the BEB.

The blood-brain barrier is believed to retard access to the CNS of diet-related ion changes in the haemolymph, for instance changes in K⁺ concentration, preventing K⁺ from depolarizing neurons and disrupting neural function. It therefore should be characterized by a high electrical resistance, the result of restriction upon the movement of small ions. Paradoxically, an earlier analysis of the transcellular route at the surface of the nerve cord revealed only a moderate extracellular resistance. By contrast, examination of the eye's barrier reveals large variations of measured resistance with depth. The results are consistent with extreme occlusion of the two parallel extracellular routes at the site of the BEB, one between glia, the other between glia and the photoreceptor axons.

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03-009

VISUALLY-GUIDED NAVIGATION BASED ON OPTIC FLOW IS IMPROVED BY RETINAL SCANNING

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Some years ago, we drew lessons from insect neuroethology and neurobiology to build a 10 kilogramme visually-guided and autonomous robot equipped with a compound eye, which could move about at a relatively high speed (about 2 body-lengths/second), avoiding obstacles while running towards a target (1,2). Meanwhile, this bionic approach generated a rich feed-back towards biology by pointing to the limitations of the natural system which had constituted the source of our inspiration (the fly). This led us to ask new and relevant biological questions.

Among the physiological spin-off of this robotic approach is the finding that, in spite of their immovable dioptrics, the fly's eyes can in some cases display 'binocular vergence' (3,4). This finding has now led us to examine the possible role of 'retinal scanning' in the context of motion perception.

There are two basic limitations of a compound eye operating on the basis of Local Motion Detectors (LMD's) and driving an agent (animal or robot) in transitory steps of finite length ΔL. These limitations are: (i) the range of vision varies in proportion to the step length (5), (ii) the range of vision becomes dramatically small around the pole of the optic flow field (heading direction). It turns out that retinal scanning can alleviate these two limitations, if the visual axes of the eye are made to rotate smoothly in the anterograde (regressive) sense during every translational step made by the agent. A controlled scanning of this kind considerably enhances the field of vision and detectability of the eye. This novel, biologically inspired principle was first validated via simulation and then implemented onboard a new experimental robot, which was equipped with (i) a single 'vertebrate' camera eye, (ii) an array of 'fly' LMD's, (iii) an electromagnetic scanner

With only 24 pixels, this robot manages to use its vision to navigate safely in an arena without bumping into the contrasted walls.

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03-011

TIGHT JUNCTIONS IN INSECT TISSUES - AN HISTORICAL OVERVIEW

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Over the years there has been an ongoing controversy as to whether or not insect tissues possess typical occluding, or 'tight' junctions. It has been clearly demonstrated that these organisms have permeability barriers, in such systems as the brain (central nervous system (CNS)), testis and eye. The precise morphological basis of the blood-brain barrier, and blood-testis barrier, has been hotly contested. Initially it was considered that the septate junctions, junctional structures unique to the invertebrates, were the physiological equivalent to the tight junctions found in vertebrate tissues and established there as the structural basis of barriers. However, unequivocal tight junctions with all the features of the vertebrate intercellular structures, were shown to occur in the modified glial cells, (the equivalent of vertebrate endothelia), surrounding the brains of the arachnids; very similar but simpler structures were observed in insect CNS and testis. In insects, there is a complex spiral arrangement of the outer glial cells of the perineurium, that ensheathes the avascular CNS. These cellular processes overlap extensively and form autocellular junctions which, although relatively infrequent, may be effective in forming a permeability barrier. This has led to speculation that the tight junctions of insects need not be so complicated in organization as those of arachnids and vertebrates. This is reflected in the observed extent and distribution of their component intramembranous fibrils in freeze-fracture replicas. Models can be constructed of their likely structure and possible mode of assembly.

03-012

THE STATUS OF THE SUPERFICIAL CELLULAR COMPONENTS OF THE INSECT NERVOUS SYSTEM.

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This presentation is dedicated to the memory of Dr Berta Scharrer, whose pioneering studies of the insect nervous system addressed a key issue for consideration in this symposium. She made a clear distinction between the connective tissue cell layer that overlies the central nervous system where it secretes the neural lamella, and the outermost investing layer of glial cells.

It is now established from studies with the twist mutant in *Drosophila* that the outermost embryonic glial cell layer can generate a functional ion barrier in the absence of mesodermal cells, and that this layer develops relatively early in embryogenesis. It is recommended that this layer be referred to as the barrier glial layer since the term perineurial glia has been loosely used to include the connective tissue cells together with the outermost glial cells.

While a single cell layer is sufficient to constitute an ion barrier in the embryo, a multilayered barrier in larger insects is not precluded.

The ion barrier is well established in terms of structure and function for the central nervous system, but the status of the barrier on peripheral nerves is in need of further study.

03-014

REVIEW ON OVARY DEVELOPMENT AND PHYLOGENY OF INSECTS

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Insect ovaries develop from a somatic mesodermal sheath, to which germ cells migrate from the posterior egg pole and settle in regions of the 3rd to the 7th abdominal segment. The somatic sheath bends into a gonadal tube and germ cells will move finally into a ball shaped or elongated ovary anlage. There, apical and basal mesodermal cells undergo further differentiation by which ovarioles will separate; i.e. insect ovarioles do not follow segmental boundaries but are produced by secondary processes inside an existing, sac shaped ovary. Within each ovariole, cluster behaviour of germ cells will raise the different types of insect ovarioles: panoistic, polytrophic meroistic and telotrophic meroistic, while germ cell - somatic cell interactions are necessary during differentiation programs of oocytes, nurse cells and follicular cells.

All insect (Ectognatha) orders have ovarioles or have lost this type secondarily due to parasitic way of life (Strepsiptera). The sister group of true insects are the Endognatha, which have all but one sac shaped ovaries, as do have Chilopoda, Myriapoda and other related groups. The exception among Endognatha are the Japygida which develop ovarioles, nearly identical to ovarioles of true insects. Thus, a revision is made by which Japygida and Insecta (Ectognatha) will form the new taxon "Ovariolata". A second revision is proposed by the raise of the basic type of the polytrophic meroistic ovary: the taxon "Meroista" includes the Dermaptera, the Acercaria and the Holometabola.

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03-013

DIPTERAN BLOOD-BRAIN BARRIER;
CURRENT FINDINGS, FUTURE PROSPECTS

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The blood-brain barrier (BBB) insures brain function in vertebrates and insects by maintaining ionic integrity of the extraneuronal bathing fluid. We have studied this important redoubt in immature dipteran (*Delia*, *Drosophila*) flies. There are key ultrastructural and functional analogs between the BBB of a fly and that of vertebrates. Septate junctions are the invertebrate functional analogs (as a blockade to tracer) to vertebrate tight junctions. Both junctional types also exhibit genetic and molecular kinships, based on the work of others. Anionic domains lining luminal surfaces of vertebrate epithelial cells are similar to a charge-selective stratum we found in the neural lamella of the CNS of the *Drosophila* larva. We find that hyperosmotic and Ca⁺⁺ chelating agents breach the BBB as they do in vertebrates. The barrier in the larva is based on occlusive septate junctions between perineurial cells, while in the embryo the first appearance of the barrier is in recently formed septate junctions between glial cells of nascent chordotonal organs in early neurogenesis. At that time the CNS is unprotected, but by Stage 17 the CNS is fully vested with a BBB. We suggest the BBB initiates in the PNS and probably accompanies PNS axons to the CNS which later gains its own barrier prior to attaining electrophysiological competence. An over-all perspective of the dynamics of the BBB from early neurogenesis to pupal life is presented. Basic studies of fly BBB may have specific import in insect toxicology; *Drosophila* may prove to be a useful animal model for BBB research in general. Supported by NSF (BNS 8908081), State Transitional Fund and Hatch Project 3857.

03-015

GERM CELL CLUSTERS AND GERM CELL ACTIVITY IN
NEUROPTERAN OVARIES.

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Precise analysis of polytrophic ovary structure in Neuroptera revealed the unusual organization of their germ cell clusters. In all species studied so far (representatives of 5 families) cystocytes divisions are asynchronous so the clusters with variable and unfixed number of cystocytes are formed. Moreover, spatial organization of cystocytes connections is not typically branched, but rather linear (only a few branching sites could be observed). The oocyte is located in the central, always linear part of the cluster and therefore is directly connected via intercellular bridges with only two nurse cells.

Linear character of germ cell clusters in Neuroptera is conditioned by asynchrony of cystocytes divisions. Mitotic activity is limited to the terminal parts of the cluster so although the number of cells may grow branching does not appear. Cessation of mitotic activity by some cystocytes enables their earlier differentiation and thus leads to an early diversification of germ cells within the cluster.

03-016

FORMATION AND DIFFERENTIATION OF GERM CELL CLUSTERS IN HYMENOPTERANS

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Oogenesis of 5 hymenopteran species (*Cosmoconus meridionalis*, *Chrysis ignita*, *Vespa germanica*, *Mutilla marginata* and *Campanotus* sp) have been analysed. Ovarioles of investigated species as those of other hymenopterans are of the polytrophic-meroistic type. Within ovarioles, terminal filaments, germaria and vitellaria can be distinguished. Germaria of social species (*Vespa germanica* and *Campanotus* sp) are additionally differentiated into 2 well defined regions. In the anterior one mitotic divisions of germ cells take place. Resulting germ cell clusters differentiate into oocytes and nurse cells in the posterior region of the germarium. Vitellaria contain egg chambers in a linear arrangement; each egg chamber is composed of an oocyte and species specific number of nurse cells. In all examined species the contact of the oocyte with accompanying nurse cells is ensured by a cytoplasmic extension of the anterior oocyte pole, termed the nutritive appendix. The apex of the appendix is markedly expanded and bears all intercellular bridges that join the nurse cells with the oocyte. Distribution of microtubules and microfilaments have been studied in oocytes and nurse cells throughout the process of oogenesis. We show that in hymenopterans, as in *Drosophila* cytoskeletal elements play a role in such processes as: differentiation of germ cell clusters, positioning of organelles within ooplasm, transportation of macromolecules from nurse cells to the oocyte. We found however, several important differences between fruit fly and our model system.

03-018

THE ROLE OF TRANSCRIPTION FACTORS IN DORSAL-VENTRAL PATTERNING DURING *DROSOPHILA* OOGENESIS

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Establishment of dorsal-ventral polarity during *Drosophila* oogenesis is initiated by transmission of a "dorsal signal" from the oocyte to the overlying anterior dorsal follicle cells via the EGF receptor pathway. As a result, this population of follicle cells undergoes changes in cell shape, cell migration, and specific gene expression. Ultimately these cells synthesize and secrete chorion proteins and contribute to the formation of specialized chorion structures such as dorsal respiratory appendages, the operculum and the micropyle. In this process, the roles of mRNA localization, ligand-receptor interactions and phosphorylation events have been studied extensively, but the final transcription-factor "targets" of the signaling pathways remain elusive. We demonstrate that CF2, a zinc-finger transcription factor, is a key mediator of these processes. CF2 is absent in the anterior-dorsal follicle cells, but present in the remaining follicle cell nuclei. CF2 expression is ubiquitous in ventralized mutant eggs (e.g. *gurken*) and the patch of CF2 absence expands in dorsalized mutants (e.g. K10). Ectopic expression of CF2 leads to ventralization of the egg, whereas loss of CF2 results in dorsalization. We propose that CF2 is a suppressor of dorsal fate. Notably, loss of CF2 leads to formation of supernumerary dorsal appendages. This suggests that CF2 alone may suppress the entire program of dorsal differentiation, at least in certain populations of follicle cells. A second goal is to understand the role of hormone-signalling during oogenesis by examining expression and action of nuclear hormone receptors. The ultraspiracle (USP) and Ecdysone receptor (EcR) gene products are both found in follicle cell nuclei throughout oogenesis. These proteins dimerize and form the functional receptor for 20-OH ecdysone. We have recently identified a new *Drosophila* hormone receptor, DHR38. This receptor is able to dimerize with USP *in vitro* and in Schneider cells, competing effectively with EcR. DHR38 mRNA is also detected during oogenesis; current work includes examining DHR38 protein expression in developing egg chambers. Interestingly, overexpression of DHR38 results in a gross expansion of dorsal material and shortened dorsal appendages with no apparent effect on ventral follicle cells. This phenotype is partially rescued by simultaneous overexpression of USP, suggesting that a direct interaction between the two proteins can occur in flies. Possible mechanisms leading to this phenotype will be discussed. Loss-of-function DHR38 mutants are currently being isolated and will be examined for egg phenotype.

03-017

THE ROLE OF THE *l(1)3Ae*-GENE PRODUCT IN CELL FATE DECISIONS DURING OOGENESIS OF *DROSOPHILA MELANOGASTER*

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We have isolated a viable allele of the *Drosophila l(1)3Ae* = *l(1)zw4* gene, named *l(1)3Ae^{em}*. The ovarian follicles of mutant females display a variety of abnormalities that affect germ cell number, follicle polarity and oocyte-nurse cell differentiation. All of these phenotypes are considered to be due to disturbed interactions between the germ line and prefollicular cells, pointing to an important role for such interactions in germ cell differentiation. The eggs produced by mutant females are never laid. Those retained eggs often have fused dorsal appendages resembling phenotypes from weak torpedo alleles (*top: Drosophila* EGF receptor homologue = *DER*). Flies mutant for both *l(1)3Ae^{em}* and *top* are extremely rare and have more severe defects in oogenesis indicating that the *l(1)3Ae* and *DER* gene products cooperate in the same pathway during oogenesis. We have generated germ cell clones in heterozygous females by employing the dominant female sterile-FLP recombinase technique (Chou & Perrimon, 1992: Genetics 131, 643). Such females lay eggs that also have ventralized eggshells in many cases; this demonstrates that the inability to lay eggs is due to a somatic defect and that *l(1)3Ae* acts in the germ line, i.e. upstream of the EGF receptor. Progress will also be presented in the molecular characterization of the *l(1)zw4* gene. Supported by DFG grants to J. B. and U. S.

03-019

EVOLUTION OF YOLK PROTEIN GENES AND THE REGULATION OF THEIR EXPRESSION IN DIPTERAN INSECTS

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In *Drosophila*, the three *yolk protein* (*yp*) genes are expressed in adult females in only two tissues: ovarian follicle cells and fat body cells. In addition, *yp* expression is limited to part of the follicle cells life time and to those follicle cells overlying the oocyte. Thus sex, tissue and developmentally-specific expression provides an excellent opportunity to understand how a complex pattern of gene expression can be achieved during development. Furthermore *yps* represent the only example of a direct link between the sex-determination pathway and the downstream sex-differentiation genes. We have used comparative evolutionary studies to elucidate the control signals for sex- and tissue-specific gene expression. We cloned the *yp* genes from *Calliphora erythrocephala* and *Musca domestica* and their analysis at the molecular level showed they are very well conserved when compared to the *Drosophila* proteins, not only in their sequence but also in their expression pattern. In order to identify the trans-acting factors binding to cis-regulatory sequences and to analyse their conservation through evolution, we took 5' flanking regions from one *Calliphora yp* gene and one *Musca yp* gene and used them as enhancers with a *lac-Z* reporter. We showed that two fragments of 823 bp and 900 bp isolated from *Musca* and *Calliphora yp* genes respectively, are able to direct correct reporter gene expression in the ovary of the transformed flies as β -galactosidase assays showed staining in ovarian follicle cells surrounding the oocyte and in the border cells at specific developmental stages. However, these enhancers do not confer sex-specificity of expression in the fat body, as reporter gene expression was found in both sexes of the transformed flies. This means that tissue specific trans-acting factors are conserved between these 3 dipteran species, while trans-acting factors conferring sex-specific *yp* expression, eg doublesex (*DSX*) are either not present or have diverged sufficiently to be non functional between species. The conservation of the ovarian regulatory pathway may enable us to identify the ovarian trans-acting proteins directing follicle cell expression. On the other hand the finding that the sex-specificity of expression is not conferred by these heterologous enhancers provides information about the evolution of sex-determination in the dipteran insects have been established suggesting that sex determination mechanisms are evolving much more rapidly than mechanisms governing tissue specific gene expression in *Musca*, *Calliphora* and *Drosophila*.

03-020

DEVELOPMENTAL CONTROL OF *DROSOPHILA* VITELLINE MEMBRANE PROTEIN GENES EXPRESSION.

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Early embryonic development depends on complex processes occurring during oogenesis which, in *D. melanogaster*, can be easily approached at the genetic, cellular and molecular levels. During late stages of oogenesis, the follicle cells are engaged in the formation of the eggshell layers: vitelline membrane and chorion. The vitelline membrane is composed of a small number of abundant proteins and several minor constituents. The transcriptional regulation of the vitelline membrane protein genes so far cloned is highly coordinated, and the pattern of gene expression for each member of the gene family has been described. We are interested in the study of vitelline membrane protein gene *VM32E* that shows a peculiar temporal and spatial regulation as compared to the other VM genes. We focused our work on a fine dissection of *VM32E* gene promoter by means of germ line transformation experiments. These analyses allowed us to identify in the region from nt -349 to nt -39 upstream the *VM32E* transcription initiation site specific regulatory elements, driving the developmental expression pattern of the *VM32E* gene in the different follicle cell subpopulations.

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03-022

CYTOSKELETON OF FOLLICULAR CELLS, CHORIOGENESIS AND EGG SHELLS OF ANOPLURANS AND MALLOPHAGANS

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Egg morphology and process of choriogenesis have been studied in the anopluran, *Haematopinus suis* and 3 mallophagans, *Eomenacanthus stramineus*, *Columbicola columbae* and *Lipeurus maculatus*. In all investigated species egg shells are equipped with anterior operculum and posterior hypople. Anterior half of the egg shell of *Eomenacanthus stramineus* is additionally covered with long, variously shaped bristles. Hypopyles always consist of parallel canals that penetrate the whole thickness of the chorion. The main egg shell of mallophagans is solid and devoid of openings. In contrast, egg shell of *Haematopinus suis* is perforated by numerous narrow canals. Described above regions of the egg shells are deposited by morphologically distinguishable subpopulations of the follicular epithelium. In addition to gross morphology, these subpopulations differ also in the distribution of cytoskeletal elements. Two chorionic structures, hypopyles and canals perforating the egg shell of *Haematopinus suis* are formed around processes of the follicular cells. These specialized structures contain numerous microfilaments. During choriogenesis of *Eomenacanthus stramineus* intercellular spaces between anterior follicular cells gradually widen. In these "empty" spaces bristles covering the anterior half of the egg shell are formed. Prior to ovulation, thick bundles of microfilaments arise in the basal cytoplasm of follicular cells. These bundles show muscle-like cross-striations and are apparently involved in the passage of the developed oocyte from the ovariole to the lateral oviduct.

03-021

TERMINAL DIFFERENTIATION OF OVARIAN FOLLICULAR CELLS IN THE SILKMOTH *BOMBYX MORI*: MOLECULAR SWITCHES CONTROLLING A COMPLEX REGULATORY PATHWAY

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Studies in our laboratory are focused on the mechanisms that control the terminal differentiation of ovarian follicular cells in the domesticated silkmoth, *Bombyx mori*. The terminally differentiated state of follicular cells is manifested by the synthesis and secretion of the chorion polypeptides that form the eggshell or chorion whose function is to allow fertilization and protect the developing embryo.

The initiation of oogenesis in silkmoths is controlled by the steroid hormone 20-hydroxy-ecdysone (20E). However, the identity of the components that constitute the regulatory cascade controlled by 20E remains largely unknown. On the other hand, the commitment of follicular cells to their terminal differentiation path occurs during mid-vitellogenesis, 1.5 days prior to the onset of choriogenesis. Commitment involves a secondary regulatory cascade that controls the synthesis of transcription factors required for activation of chorion genes. Our hypothesis is that the primary regulatory cascade that is controlled by 20E culminates in the appearance of a receptor for a circulating ligand in follicular cells. The ligand for this receptor may be a member of the bombyxin family of insulin-like peptide hormones, that are present in the circulation of female (and male) pupae at a time coinciding with the time of commitment of follicular cells to terminal differentiation.

To deduce the validity of our model, we have cloned an isoform of the receptor for 20E (BmEcR), that is expressed in follicular cells, confirmed its identity by a number of functional assays, and analyzed its distribution in follicular cells during oogenesis. Furthermore, work is in progress to achieve three additional objectives: (1) the isolation of cDNA sequences expressed during the earliest stages of oogenesis (the phase preceding the commitment stage) and analysis of their genes to deduce whether the latter are regulated directly by the 20E/BmEcR complex; (2) the cloning of sequences expressed in follicular cells after the establishment of the choriogenic potential (*i.e.*, after commitment has occurred) but prior to its implementation (choriogenesis); and (3) the cloning of the bombyxin receptor(s) that may act in follicular cells as the switch for the second regulatory cascade.

03-023

THE MORPHOGENESIS OF THE EGG CHORION IN *DROSOPHILIDAE* (DIPTERA). A MODEL FOR THE ORGANIZATION OF COMPLEX SUPRAMOLECULAR STRUCTURES IN EUKARYOTES.

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Chorion in *Diptera* is a complex proteinaceous extracellular structure exhibiting regional and radial complexity that finally assembles in a functional supramolecular structure. This includes, a crystalline innermost chorionic layer (ICL), an endochorion with inner part (floor), pillars and outer part (roof) and an exochorion. The chorion proteins are crosslinked in vivo via the action of a chorionic peroxidase (CP) upon activation by endogenous hydrogen peroxide. Besides its use as a model system in developmental biology, the insect chorion is of great importance to answer questions of evolutionary biology, molecular genetics, supramolecular architecture and physiology.

We have recently focused our research on chorion morphogenesis in *Drosophila virilis* for which there is an increasing accumulation of genetic information. Its chorion is comprised of six major proteins that exhibit a distinct temporal synthetic profile and have been characterised as early (Dvs38, Dvs36), middle (Dvs19, Dvs16) and late (Dvs18, Dvs15) according to the choriogenic stage they are produced. In order to explore the way that the chorion is assembled, specific antibodies have been raised, against the two early, one middle and one late chorion proteins. These antibodies have been used for immunobiochemical and immunocytochemical studies and have revealed that:

a) the chorion proteins are randomly distributed within the endochorion, but they are absent from the ICL. b) all specialised regions (appendages, operculum, micropyle, collar, posterior pole) have been shown to contain all proteins. c) the early proteins alone are organising a structurally complete endochorion. The subsequently secreted middle and late proteins are intercalating amongst the early protein molecules. e) double immunolocalization has shown that the chorion proteins do not coexist in every secretory vesicle, suggesting that the final rearrangement of the protein molecules probably occurs extracellularly.

These results allow us to conclude that during chorion formation a scaffold is first formed consisting mainly of the two early chorion proteins, which is subsequently filled by the middle and the late chorionic proteins. Therefore the system exists in a dynamic equilibrium until the moment that an unknown agent (probably hydrogen peroxide which acts as an activator of the peroxidase) causes the final rearrangement of the components resulting in the hardening of the structure. Furthermore, the fact that these major proteins appear to participate in all the specialised regions, suggests that the structural variability of these regions is most probably due to differential secretory pattern by the corresponding follicle cell subpopulations.

03-024

OOTAXONOMY IN PHLEBOTOMINE SANDFLY (DIPTERA, PSYCHODIDAE).

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Insect ootaxonomy, based on egg chorionic sculpturing observed by scanning electron microscopy, is well advances for various families of the dipteran order. Among phlebotomine sandflies, many of which are medically important vectors of leishmaniasis and Phleboviruses, the eggs of several species belonging to the Neotropical genera *Lutzomyia* and *Psychodopygus* have been previously examined by SEM.

Recently, we have investigated, by means of scanning (SEM) and transmission (TEM) electron microscopy, the eggshell fine structure of species belonging to the Palearctic genera *Phlebotomus* and *Sergentomyia*. At TEM level, the eggshell appears to have a homogeneous vitelline envelope and a thick chorion. The latter is formed by distinct inner, intermediate and outer layers. At SEM level, the outer chorionic layer shows a characteristic pattern, consisting of small columns arranged in palisade to form sinuous ridges.

On the basis of the chorionic sculpturing, the eggs of the sandfly species were grouped into different morphological categories.

SEM/TEM investigations of the sandfly eggshell show species-specific characters useful for their specific identification. The chorionic features of different species are compared with those of other Diptera and their phylogenetic value is discussed.

03-026

COMPARATIVE MORPHOLOGY OF SENSILLUM DEVELOPMENT

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Each insect sensillum is formed by a fixed number of cells which arise via differential mitoses from an epidermal mother cell. One or several bipolar sensory neurons are enveloped by the glia-like thecogen cell, the trichogen, and the tormogen cell. Morphogenesis begins with formation of an apical ciliary (9x2+0) process by the neuron which grows above the epidermal surface. This is followed by the outgrowth of an apical sprout, backed by a microtubular cytoskeleton, from the trichogen cell, which will secrete the cuticle of the sensory hair. The tormogen cell forms the hair socket. After cuticle deposition, the trichogen cell retracts from the hair shaft and, together with the tormogen cell, forms the subcuticular sensillum lymph space.

In hemimetabolous insects in preparation for molting, the dendrite leaves the newly developing sensillum through a lateral or apical pore, remaining connected with the old sensillum. This feature is conserved in holometabolous insects, where during adult development the dendrites also project from the newly forming sensilla although the pupa does not have sensilla to which the dendrites could be connected. When cuticle secretion begins, these dendritic processes are lost. The definite sensory dendrites grow into the hair shaft while the trichogen cell retracts from the latter in most sensilla investigated so far. In many olfactory sensilla, the tormogen cell degenerates at the same time and is replaced by an additional fourth enveloping cell.

03-025

DOES CHORIONIC STRUCTURE PLAY A ROLE IN THE ADAPTIVE RADIATION OF THE HAWAIIAN *DROSOPHILA*?

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The endemic Hawaiian *Drosophila* have long been recognized for their extraordinary divergence in external adult morphology, but even more diversity is found in egg size and ultrastructure of the chorion. Among about 100 Hawaiian species analyzed thus far, no two species share identical chorion morphology. Nonetheless, chorion structures in taxonomically related species are more similar than those of different species groups or subgroups, suggesting a genetic basis for the morphological differences and the possibility of tracing phylogenetic trends in chorion morphology. It is hypothesized that much of the divergence in chorion morphology has evolved in response to varied selection pressures in the ecologically diverse breeding substrates used by Hawaiian drosophilids. This fauna has radiated to oviposit in fungi, decaying flowers, fruits, leaves, stems, bark or tree fluxes, these diverse substrates imposing varied demands on the oviposition process and survival of the embryo. To investigate correlations between aspects of chorion morphology and the oviposition substrate we have taken a phylogenetic approach, using an independently derived molecular phylogeny of the Hawaiian species to conduct analyses of chorion characters such as the length of the respiratory filaments, thickness of the outer endochorion, and features of the follicle imprint borders, dorsal ridge, operculum, micropyle, etc. These data allow us to examine whether the evolutionary divergence in chorionic characters has an adaptive basis.

03-027

Genes involved in the development of sensory organ precursors in Drosophila.

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The genetic control of sensory organ development is being unravelled in *Drosophila*. During development of the peripheral nervous system, the potential to become nerve cells is provided by the genes *achaete* (*ac*) and *scute* (*sc*). They encode bHLH transcription factors. The pattern of bristles on the thorax results from a complex and dynamic expression of *ac* and *sc* at the sites of each future sensory organ. Control of the spatial expression is not understood but several upstream regulatory genes transcription factors are known. These are expressed over part of the thoracic epithelium and have been shown to regulate expression of *achaete-scute* within their domains of expression. *achaete* and *sc* are expressed initially in clusters of cells from which one cell is singled out to be a neural precursor. This cell then prevents the other cells of the cluster from becoming neural by means of an inhibitory signal that results in the cessation of *ac-sc* expression. This is known as lateral inhibition. The signal is mediated by *Delta* that acts as a ligand for the *Notch* receptor. A loss of any one of the components of this signalling cascade results in neural hyperplasia: all of the *ac-sc* expressing cells, instead of just one, follow the neural pathway. A number of genes are also known that act after *ac* and *sc* and play a role in the determination of sensory organ type. Finally the genetic control of the assignment of cell type among the four cells composing the bristle organ has also been partly described.

03-028

COMPARISON OF INSECTS AND CRUSTACEANS
MECHANORECEPTORS
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Mechanosensitive cells of crustacean setae show important cytological differences compared with those of insects; the mechanosensitive cells of arthropods setae are classified in two groups which are structurally and functionally different; the typical cells of insects setae show a tubular body at the tip of their dendrites (tubular body cell: **tbc**); mechanosensitive cells of aquatic crustaceans never show such a structure and are characterized by a 9+0 cilium and a large ciliary root (chordotonal type cell: **ctc**); among crustaceans, **tbc** are only observed in terrestrial isopods. Therefore, it seems that the type of mechanosensitive cell associated with an arthropod seta is more linked to the physical environment than to the arthropod class. It is also linked to differences in the kind of mechanosensitivity of the two types of cells: **tbc** dendrites would be compression sensitive whereas **ctc** dendrites would be stretch sensitive. The presence of **tbc** in the aerial medium and of **ctc** in the aquatic medium could be explained by the physical characteristics of these two media.

03-030

FUNCTIONAL ANATOMY OF ANTENNAL MULTIPOROUS
GUSTATORY SENSILLA IN SOME PARASITOID
HYMENOPTERA

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Multiporous gustatory sensilla (MGS) present on females antennae of parasitoid Hymenoptera have been investigated and compared in some species belonging to Scelionidae (*Mantibaria anomala* Kirby, *Telenomus busseolae* Gahan, *Trissolcus basalis* Woll.), Platygastridae (*Amitus spiniferus* Brethes), Trichogrammatidae (*Trichogramma brassicae* Bezdenko) and Diapriidae (*Coptera occidentalis* Mues., *Trichopria* sp.). They are located ventrally with number and pattern remarkably variable in different taxa. In Scelionidae and Platygastridae the MGS are located in median longitudinal row, in small number (3-9/antenna), and innervated by very numerous sensory neurons (120-400/sensillum). Instead in Trichogrammatidae and Diapriidae the MGS are scattered in relatively high number (20-240/antenna) and innervated by few sensory neurons (5-10/sensillum). Outer dendritic segments, usually unbranched or branched in one case, reach the multiporous area which presents peculiar features in different taxa. Accessory glands are in some cases associated with MGS. Their possible role in host recognition is discussed.

03-029

STRUCTURAL AND TOPOCHEMICAL ANALYSIS
OF OLFACTORY SENSILLA IN MOTHS

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The pheromone detection system of male moths was used for a long time as a model in insect olfaction. Milestones were the discovery of elementary receptor potentials, second messenger cascades, and odorant-binding proteins. Topochemistry, in particular immunocytochemistry, has become a link to combine biochemical data, usually obtained from homogenized antennae, with the complex and diverse ultrastructure of antennal sensory organs.

Specific antibodies against odorant-binding proteins (OBPs) and putative key proteins in the transduction machinery now allow for a localization of these proteins on the ultrastructural level. Immunolocalization of pheromone-binding protein (PBP) and general-odorant binding protein (GOBP) in *Antheraea polyphemus*, *Bombyx mori* and other moth species revealed that these proteins are highly concentrated in the aqueous sensillum lymph, which surrounds the dendritic portions of the receptor cells. PBP is predominantly expressed in pheromone-sensitive sensilla trichodea of male moths, whereas GOBP could be detected in sensilla of both sexes, which are sensitive for plant odours and other 'general odours'. Odorant-binding proteins of different classes (PBP and GOBP) are not co-expressed within a given sensillum. The expression of a certain OBP is not correlated with a certain morphological sensillum type but rather with the specificity of the olfactory receptor cells contained. These results favour the hypothesis that OBPs might play a role in stimulus recognition in addition to their transporter and deactivator function.

We also succeeded in the localization of several proteins of suggested significance in the second messenger chain. A G-protein of the Gq-family could be localized in the receptive dendrite of the receptor cells as well as an IP₃ receptor. In addition, Calmodulin and Calcineurin were detected in the olfactory receptor cells. Nitric oxide synthase was found to be highly concentrated in the antennal haemolymph. This enzyme produces the messenger NO which is possibly involved in receptor adaptation via guanylate cyclase. Comparison of the stimulated versus unstimulated state of olfactory sensilla may add further insight in the functional significance of these proteins.

03-031

PERCEPTION OF INFRARED RADIATION BY SPECIALIZED
THERMORECEPTORS IN BUPRESTID BEETLES

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Larval development of buprestid beetles of the genus *Melanophila* can take place only in trees recently killed by a fire. For the localization of forest fires the adult beetles have metathoracic infrared pit organs next to the coxae of the mesothoracic legs enabling both sexes to detect infrared radiation at long range. Dependent on the size of the beetle, at the unsclerotized bottom of each pit 50 - 100 sensilla can be found. The cuticular apparatus of a single sensillum consists mainly of a massive endocuticular spherule of 12 - 15 µm in diameter. The upper hemisphere of the spherule bulges out and is covered only by a thin mesocuticle of about 1 µm. Except for a small cuticular stalk (diameter 2 µm) connecting the spherule to this outer cuticle, the entire spherule is surrounded by distal processes of two enveloping cells (probably the tricho- and the tormogen cell) forming a flat protoplasmatic double layer of about 300 nm thickness. Therefore the upper region of the outer receptor lymph cavity remains comparatively small. From below the spherule is innervated eccentrically by the distal tip of the dendritic outer segment (DOS) of a single ciliary receptor cell. In the outermost tip of the unbranched DOS, tightly squeezed in the spherule, a well developed tubular body can be found. Apart from the peculiarities of the cuticular apparatus the remaining ultrastructure of the sensillum is highly reminiscent to that one of a cuticular hair mechanoreceptor (sensillum trichodeum). Because transitional stages between infrared receptors and sensilla trichodea can be found and in the posterior slope of the pit so called suppressed infrared sensilla are situated which remain completely below the cuticle and display spherules which are basally flattened and longitudinally stretched, it is postulated that the infrared receptors must have evolved from cuticular mechanoreceptors.

03-032

FUNCTIONAL ROLE OF CYTOSKELETAL ELEMENTS IN INSECT SENSILLA

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Insects gather information about environmental conditions using special sensory organs, the sensilla. As has been demonstrated in mechanosensitive structures of other organisms, mechanosensitive sensilla of insects possess prominent cytoskeletal elements with characteristic micro-mechanical properties. These properties may play important roles in sensilla function. To get more insights into the micro-mechanics of sensilla, the molecular composition of the cytoskeletal elements in the sensory cells, as well as the auxiliary cells, has been analyzed.

1. The scolopale in the innermost auxiliary cells of the mechanosensitive scolopidia and thermo-hygroresponsive no-pore sensilla are comprised mainly of actin filament bundles. Their filament orientation and the presence of actin-associated proteins (e.g. tropomyosin) indicate that these actin bundles stabilize the scolopale, and their characteristic elasticity is probably involved in stimulus transformation in scolopidia.
2. An additional cytoskeletal component of scolopidia is the prominent ciliary rootlet of the sensory cell. Immunocytochemistry shows that these rootlets are composed of centrin, a small Ca^{2+} -binding protein which forms contractile filaments in green algae. Rootlet contraction in scolopidia may serve in sensory adaptation.
3. In mechanosensitive cuticular sensilla, a prominent cytoskeletal structure - the tubular body - is localized at the tip of the dendritic outer segment of the sensory cells. Present results reveal that the microtubules composing the tubular body are linked to each other by microtubule-associated proteins. This stabilization confirms the hypotheses that the tubular body is forming a rigid core underlying the mechanosensitive membrane of the tip of the sensory dendrite.

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03-034

SPERM AND EGG SURFACE COMPONENTS POTENTIALLY INVOLVED IN GAMETE INTERACTIONS IN *Drosophila melanogaster*.

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In a variety of species sperm-egg recognition involves interactions between glycoconjugates and complementary molecules present on the gamete surfaces. Using cytochemical approaches and biochemical assays, we studied the presence of carbohydrate residues and complementary molecules on the surface of the gametes of *Drosophila melanogaster*.

In the chorion, residues of αMan , αGalNAc and βGal are present on the whole surface of mature ovarian eggs, whereas βGlcNAc , αGal , $\alpha\text{L-Fuc}$ and Neu5Ac residues are restricted to the micropyle. The vitelline envelope (VE) shows a high density of αMan and βGlcNAc moieties in its micropylar portion, over the site of sperm entry. Upon fertilization, no modification in the distribution of the surface sugars occurs in the chorion, whereas αMan and βGlcNAc are no longer detectable on VE.

In sperm of fertile males, binding sites for αMan and βGlcNAc are present on specific domains of the plasma membrane overlying the middle portion of the acrosome and the axoneme terminal tract. Fluorimetric assays indicated the presence on the sperm surface of $\beta\text{-N-Acetylglucosaminidase}$ ($\beta\text{GlcNAc-ase}$) and of $\alpha\text{-mannosidase}$ activities with a pH optimum of 3.9.

In sperm of the male autosomal sterile mutant *ms(3) HB156* that are motile and are transferred to the female but that do not enter the egg, binding sites for βGlcNAc are absent from the acrosomal tract of the plasma membrane and surface $\beta\text{GlcNAc-ase}$ activity is 50% of that present on sperm of fertile males. Binding sites for αMan and $\alpha\text{-Mannosidase}$ activity were as in spermatozoa of fertile males.

The results suggest that $\beta\text{-N-Acetylglucosaminidase}$ on the sperm plasma membrane and βGlcNAc residues on the vitelline membrane play a crucial role in sperm-egg interactions.

03-033

Transcriptional and translational control of gene expression during *D. melanogaster* spermatogenesis: the *Mst23Ea* gene expression as a model.

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The genetic program leading to the sequential transformation of cellular components during *Drosophila melanogaster* spermiogenesis is achieved at least by two temporally distinct levels of gene expression. Most of the genes required for spermiogenesis are transcribed in the growing phase of the premeiotic spermatocytes several days before their protein products are necessary. Transcription ceases just before meiosis and the successive differentiation program, stored in pretranscribed mRNA molecules, is then controlled at translational level in absence of transcription. In order to gain further insights into the molecular mechanisms regulating this peculiar gene expression of the *D. melanogaster* spermatogenesis we undertook the study of a newly identified *Mst23Ea* gene which is exclusively transcribed in the male germline. Here we report the identification and the study of the cis-regulatory sequences directing the spermatocyte specific transcription of the gene and the postmeiotic spermatid translation of its transcripts. We show that a region from -254 to +27 bp, sufficient for a high level of proper transcription in the male germ line, can be split into two regulatory elements. A first element encompassing the transcriptional start site from -33 to +33 bp is sufficient to guarantee stage and tissue specific transcription of the gene although at a low level. A second element located from -198 to -34 has a quantitative effect on the testis specific expression. The *Mst23Ea* transcript, first synthesized in primary spermatocytes, is translated only in the postmeiotic stages. Sequences localized in the 5' untranslated leader are sufficient to repress translation of the *Mst23Ea* transcripts in the spermatocytes and early spermatids during sperm differentiation. Band shift experiments show that all the *Mst23Ea* regulatory elements bind specifically to protein factors extracted from adult testis.

We are currently investigating the functional role of the *Mst23Ea* gene product using a transgene encoding *Mst23Ea* antisense RNA, *in situ* antibody staining of the endogenous *MST23Ea* protein and *in vivo* localization of a *MST23Ea*- β -galactosidase fusion protein.

03-035

AXONEMAL DIVERSITY IN INSECT SPERMATOOZOA

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Most animal spermatozoa have a flagellum with a 9+2 pattern (i.e. 9 microtubular doublets around 2 central singlets) or a 9+9+2 one (accessory tubules flank the doublets). The A-tubule of the doublet usually has outer and inner dynein arms. However, outer arms are missing in Ephemeroptera and Trichoptera. Inner arms are missing in spermatozoa of several cecidomyiid Diptera, which nevertheless are motile. When both sets of dynein arms are missing the sperm tail is immotile. Accessory tubules presumably have a supporting role only; their cross-sectional appearance usually is circular, although in Psocoptera+Mallophaga+Anoplura it is elliptic. An intertubular material fills much of the spaces between the accessory tubules (except in Ephemeroptera). In most species the accessory tubules have a wall with 16 protofilaments but in Diplura, Ephemeroptera and Psocodea there are 13, in Phasmida 17, in Trichoptera 16-20, and in Diptera 13, 14, 15 or 16 ones (Dallai & Afzelius, 1990, 1994). The number of doublets may also vary: some species of Cecidomyiidae thus have 10 and 20 doublets in a ring and in Sciaridae and some Cecidomyiidae the number may be even higher. The bizarre sperm flagellum of Thysanoptera is derived from three 9+0 axonemes (Bode, 1983), which merge to form an asymmetric structure consisting of 9 doublets without dynein arms, 9 doublets with dynein arms, and 9 singlet microtubules also with dynein arms. (Dallai & Afzelius, 1991).

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03-036

THE ULTRASTRUCTURE AND BIOCHEMICAL CHARACTERIZATION OF AXONEMAL DYNEIN FROM *ASPHONDYLIA RUEBSAAMENI* KERTESZ (DIPTERA: CECIDOMYIIDAE).

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The aberrant structure of *Asphondylia ruebsameeni* Kertész sperm axonemes has been studied by both conventional electron microscopy and quick-freeze, deep-etching techniques. In this species more than 2500 microtubular doublets are disposed in double parallel spirals coiled around an axial core of mitochondria; each doublet is provided with outer arms only. Such a peculiar axoneme shows a bizarre pattern of movement when observed within the deferent duct of adult males. The three-dimensional structure of the dynein arms has been elucidated by quick-freeze, deep-etching preparations of demembranated unfixed sperm-cells. At first glance, the dynein arm shows a domain organization similar to that previously observed in other species of both invertebrates and vertebrates, consisting of a head region formed by two globular domains in close vicinity of the A subtubule and a thin stalk contacting the B subtubule of the adjacent doublet. However, a more accurate analysis of micrographs revealed the presence of a globular extra-component regularly intercalated between the arms.

Outer arms could not be extracted by the high salt treatment usually efficient in the axonemes of other species, and could be removed only by dialysis against a low ionic strength buffer. The high molecular weight polypeptide complement of the solubilized dynein was shown by SDS-page to consist of only one band at ca.300kDa. A polyclonal antibody specific for a synthetic peptide which corresponds to the consensus sequence of the P-loop of both axonemal and cytoplasmic dyneins strongly reacted with this polypeptide. Solubilized dynein exhibited a sedimentation coefficient of about 12S and a Mg²⁺-dependent ATPase activity which was inhibited by vanadate.

Our preliminary data suggest that *Asphondylia* sperm dynein could be the first model of a two-headed axonemal dynein in which the two heads are formed by the same polypeptide, a feature commonly found in cytoplasmic dyneins.

03-038

MORPHOLOGICAL CHANGES OF EUPYRENE AND APYRENE SPERM WITH TIME AFTER MATING IN THE FEMALE REPRODUCTIVE ORGANS OF THE BUTTERFLY *ATROPHANEURA ALCINUS* KLUG (LEPIDOPTERA: PAPILIONIDAE)

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The male and female butterflies, *Atrophaneura alcinous*, were mated by the hand-pairing method. To examine the sequence of sperm passage, samples were dissected out from male and female reproductive organs with time from the onset of mating. They were observed under a microscope after vital staining with fluorescent dye and under an electron microscope after ultrathinsectioning.

Spermiogenesis occurred simultaneously within a cyst in the testis. Eupyrene sperm bundles migrated into the vas deference, whereas apyrene sperm were not in a bundle and moved separately with each other. Thereafter apyrene sperm preceded in the pathway of the female reproductive organs. The cyst wall of eupyrene sperm bundles became loose during the passage through the vas deference and the mass of eupyrene sperm was transferred to the bursa copulatrix about 40 minutes after mating. About 6 hours after mating only apyrene sperm appeared in the spermatheca (legena receptaculi) and eupyrene sperm followed them 1 hour later. The latter were deprived of their sheath. The adult female began oviposition at the next day after mating. Such a time lag of passage between eupyrene and apyrene sperm observed in *Atrophaneura alcinous* is characteristic among Papilionidae species. Our observation suggests that apyrene sperm play a role as pilots to assist the transfer of eupyrene sperm.

03-037

SPERMATOGENESIS OF DIAPAUSE AND NON-DIAPAUSE PUPA IN THE SWEET POTATO HORNWORM, *AGRIUS CONVULVULI* (L.) (LEPIDOPTERA: SPHINGIDAE).

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Spermatogenesis was investigated in both diapause and non-diapause individuals of *Agrius convolvuli*. To determine the timetable of spermatogenesis, testes were dissected out from larva, pupa and adult, and observed under the optical microscope after vital staining with fluorescent dye and under the electron-microscope after ultrathinsectioning. Spermatocytes destined to either nucleate (eupyrene) or anucleate (apyrene) spermatozoa were recognizable in the testis of 5th instar larva.

In non-diapause individuals, larvae were reared at 25°C and 16L-8D photoperiod; their pupal stage extended for 15 days at 25°C and 12L-12D photoperiod. The eupyrene metaphases were seen during fifth instar larvae and lasted until about day-5 pupae. The earliest spermatids appeared in day-2 wandering larvae, about 3 days before pupation, however, the nuclei of these spermatids started elongation after pupation. The eupyrene spermatids with elongated nuclei, nearly mature sperm bundles, appeared at the day of pupation. The apyrene spermatocytes were seen during fifth instar larvae, and early apyrene spermatids appeared only after pupation. On the other hand, diapause was induced by rearing at 23°C and 12L-12D photoperiod; their pupae remained in diapause for 121 ± 38 days at 25°C and 12L-12D photoperiod. The timetable of the early stages of spermatogenesis was almost similar to that found in non-diapause ones. But the eupyrene metaphases continued to appear uninterruptedly throughout diapause. The eupyrene spermatids with both spherical nuclei and elongating nuclei were also seen in the diapause pupae, and the sperm bundles almost disappeared 40-60 days after pupation. In testes of diapause pupae, spermatocytes were conspicuously characterized by degenerating structures of mitochondria losing cristae, small lobed cytoplasm and concentric layers of endoplasmic reticulum around the nucleus.

03-039

SPERM RELEASE BY AN INTRATESTICULAR MUSCULAR MECHANISM IN THE TROGID BEETLE *OMORGUS FREYI*

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Desert beetles of the genus *Omorgus* display an unique mechanism of sperm release involving intratesticular visceral muscles. Like in other insects, the testes are made of finger-like follicles containing clusters of spermatogenic cells that develop synchronously; each cluster is confined within a cyst. However, in contrast with follicles of other insects, those of *Omorgus* are subdivided into sectors by radial cellular septa converging into a centrally located vas efferens. In transverse sections, they resemble oranges sliced through their equatorial plane. Further, an extended net of myofibrils surrounds the cysts, runs within the septa and attaches to vas efferens and follicular walls. Apparently, during *Omorgus* phylogenesis, the vas efferens changed its position from outside to inside the follicle. Accordingly, the myofibril net may derive from the muscular system of the intra-follicular vas efferens. In a typical follicle, sperm release is related to pressure exerted by new cysts generated at its distal end. Appearance of the novel muscular mechanism may be related to the disproportionately large size of *Omorgus* follicles; the pressure would be insufficient for sperm release if the vas efferens remained outside the follicle.

03-040

THE DORSAL VESSEL OF INSECTS: MORPHOLOGICAL SOLUTIONS TO CIRCULATORY PROBLEMS.

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Insects have an open circulatory system and the hemolymph is not needed to transport oxygen. Nevertheless, it is vital that the hemolymph reach all of the tissues of the enveloping epidermis and internal organs and tissues. To accomplish this task in the extremities, insects have evolved ingenious specializations reported by others in this symposium. However, there are major specializations of structures in the main part of the abdomen of orthopteroid insects to ensure local circulation in the perineural and pericardial sinuses. Two very different types of these specializations have evolved in locust and cockroach to accomplish local circulation that make the cardiac physiology of locust and cockroach unique. Similar specializations in other insects have not been studied in the same detail, but are equally as important.

03-042

STRUCTURE AND FUNCTION OF THE ANTENNA-HEART OF *PERIPLANETA AMERICANA*

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In insects, specially accessory pulsatile organs were developed for supplying the antennae with haemolymph. The best investigated one is the antenna-heart of *P. americana*.

A transverse muscle band connects the two ampullae, which are dilated by contracting of this muscle. The rhythm of this dilator muscle is caused by a myogenic automatism and is influenced neuronally via the nervus cardioantennalis. All muscle fibres cover the whole muscle and branch at the ends near the ampullae. Intercalated discs are missing, but the fibres are electrically coupled with an appropriate coupling coefficient. Extensively branched muscle fibres of an accessory dilator muscle are integrated in the transverse muscle. All muscle fibres revealed similar electrical properties. Electrical stimulation of the antenna-heart nerve resulted in a complex effect: a transient heart block followed by an increase in the heart beat rate. The acceleration is caused by the peptide transmitter proctolin, shown (see below) and detected biochemically. Octopamine is the inhibitory neuromediator in the antenna-heart.

Furthermore, by means of the immunofluorescence technique the biogenic amines octopamine and 5-HT as well as the insect neuropeptides proctolin and allatostatin could be shown in the antenna-heart. Colocalization of proctolin and allatostatin proved in the antenna-heart as well as in the suboesophageal ganglion (SOG) and Co^{2+} -backfills of the antenna-heart nerve suggest the SOG to be the source of this immunoreactivities.

03-041

IDENTIFIED NEURONES INNERVATING THE HEART OF INSECTS

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The number of identified insect neurones involved in the generation of behaviours such as flight, walking, respiration and stridulation, as well as neurones involved in sensory integration of visual, acoustic, olfactory and mechanical stimuli has grown so enormously that databases, or at least unified rules for labelling individual cells are urgently needed. This situation is contrasted by the fact that only very little is known about neurones innervating insect visceral organs such as heart, gut and glands. Although it has been known for a long time that the heart of insects receives innervation from either the retrocerebral glandular complex and/or segmental heart nerves from abdominal ganglia, the neurones forming these nerves are yet unknown in most insect species. Only recently some progress has been made for at least one insect species, the migratory locust. Quite a number of neurones have been identified individually which innervate the heart in these insects. The neurones show a wide spectrum of morphological and neurochemical characteristics, and almost each ganglion of the ventral nerve cord appears to contribute to the innervation of the heart.

03-043

WING CIRCULATORY ORGANS OF INSECTS - MORPHOLOGY AND EVOLUTION

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In addition to the dorsal vessel, insects possess auxiliary pumps for hemolymph supply of body appendages. The accessory pulsatile organs for hemolymph transport in the wings are located beneath the tergite of each winged segment. They suck hemolymph from the posterior wing veins through cuticular tubes running along the tergites. All wing circulatory organs consist of a pump casing, formed by the scutellum, and an associated myofibrillar pulsatile component.

The comparative investigation of more than 100 species from almost all insect orders revealed a remarkable diversity in the anatomy of the pulsatile components. They can be formed either by modifications of the dorsal vessel or by muscular diaphragms which are attached to, or separate from, the dorsal vessel. In several lineages of Holometabola convergent evolutionary trends can be reconstructed leading from dorsal vessel modifications to separate unpaired or paired pulsatile diaphragms ("wing-hearts"). There is strong evidence that the pulsatile diaphragms are individualized parts of the dorsal vessel wall. The diversity and systematical distribution of wing circulatory organs in Lepidoptera allows the reconstruction of the transformation from attached diaphragms to separate wing-hearts without postulating intermediate states of uncertain functionality.

The results lead to a new interpretation of the evolution of the circulatory organs in the thorax. Spatial constraints resulting from modifications of the flight apparatus are discussed as possible causes for the multiple parallel evolution of wing-hearts.

03-044

HOW OTHER MEMBERS OF THE FAMILY TRANSPORT SOLUTES - FUNCTIONAL MORPHOLOGY AND PHYSIOLOGY OF THE CIRCULATORY SYSTEM OF SPIDERS

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The circulatory system of most spiders consists of the heart, pericardium, arterial vascular system, the open venous system, and lung veins connecting book lungs to the pericardium. Morphological details of the heart and pericardium allow them to function co-operatively as a pressure-and-suction pump, which generates a continuous perfusion of the book lungs both during systole and diastole. The arterial vascular system and the venous open system show adaptations to cellular oxygen requirements. At least in the legs, capillarization is absent and gas exchange with the tissues has to take place primarily along the open portion of the circulatory system. The high pressure generated in the prosoma during locomotor activity is not transmitted to the opisthosoma. A neuronally controlled occlusion mechanism (muscular valve) at the anterior end of the pedicel is most likely responsible for the maintenance of this large pressure gradient. The prosomal perfusion is interrupted during fast locomotion, the opisthosomal perfusion is maintained. Cardiac output in the tarantula can be increased by two mechanisms: increase of stroke volume and increase of heart frequency. Both are related to an increase of heart pressure. Especially during the early phase of recovery from fast locomotion, there are changes in blood flow distribution. A larger share of blood is pumped to the prosoma. The underlying mechanism could be a neuronal control of the cardio-arterial valves of the opisthosomal lateral arteries.

03-046

HEARTBEAT AND REVERSAL OF HEARTBEAT DIRECTION INTERACTS WITH DISCONTINUOUS CARBON-DIOXIDE RELEASE IN RESTING PAPILIONID-PUPAE

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In non-diapausing pupae of the papilionid species *Troides rhadamantus* and *Ornithoptera priamus* (Lepidoptera, Papilionidae) within the initial half of development, discontinuous carbon-dioxide release was found to occur at the same time when heartbeat direction changes from backward pulsations to forward pulsations.

Heartbeat patterns consisted of continuous forward-pulse periods, continuous backward-pulse periods and intermittent backward-pulse periods when heartbeat and heartbeat-pauses alternated.

Thermo-anemometric, infrared-optic and ultrasonic-doppler studies indicated that the haemolymph transport in anterior direction is more effective than in posterior direction. This is due to the higher heartbeat-frequency and heartbeat-amplitude of the forward-pulsations.

A possible role of the interaction between heartbeat-reversal and carbon-dioxide release can be found in the more effective mixing of the haemo-lymph during forward-pulsations. This convective mixing shortens the diffusion pathway of carbon-dioxide within the haemolymph and so facilitates the carbon-dioxide transfer from haemocoel into tracheal space.

03-045

DISCONTINUOUS CIRCULATION AND ITS EFFECT UPON TRACHEAL VENTILATION IN ADULT INSECTS AND THE STRUCTURAL ADAPTATIONS.

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In resting adult insects (Lepidoptera, Coleoptera, Diptera and Hymenoptera) hemolymph oscillates between anterior body and posterior body, due to its internal partitioning and discontinuous heartbeat activity (periodic heartbeat reversal and/or heartbeat pauses combined with discontinuous backward flow in the perineural sinus). Heart ostia and partitioning structures behind the waist are adapted to this mechanism in different ways within different insect orders. The relatively uncomplicated circulation mechanism of higher flies with a dominant central heart chamber contrasts to the mechanism in moths where three pumping organ systems (abdomen wall, heart and accessory pulsatile organs) work in a hierarchical order. While in the resting and running blowfly air sac ventilation is driven mainly by periodic hemolymph shift alternately in anterior body and abdomen due to heartbeat reversal, in various insects intermittent abdominal pumping movements are performed and are coordinated with specific phases of the heartbeat periodicity, most frequently coinciding with the moment of maximum hemolymph accumulation in the abdomen.

03-047

CARDIAC AND RESPIRATORY ACTIVITIES IN THE ADULT FLY, *PROTOPHORMIA TERRAENOVAE*

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In adult flies, cardiac activity consists of the regular alternance of a fast phase with a slow phase, at higher and lower beating frequency respectively. A periodical inversion in the direction of haemolymph flow corresponds to phase alternance, which is thought to play an essential role in tracheal ventilation. In this study we analyzed electrocardiographic activity in intact and semi-intact preparations, to determine the most significant heart activity parameters in adult flies. We also attempted to see if any relationships exist between cardiac and respiratory activities. To this end, heart activity and endotracheal pressure were simultaneously monitored on intact specimen by means of electrophysiological and piezoelectrical techniques respectively. Changes in abdominal sizes during regular heart cycles were also measured by means of a computerized image analyzer system. Results showed that bioelectric heart activity consists of impulses propagating forwards along the vessel during the fast phase and backward during the slow phase. Consequently, haemolymph flows towards the head during the fast phase and towards the abdomen during the slow phase. Cardiac and respiratory activities are closely related, showing cyclic synchronous time courses. A deep inspiration occurs simultaneously with an abdominal expansion, which takes place just before the beginning of a fast phase. A profound expiration occurs simultaneously with an abdominal retraction, which takes place immediately before the beginning of a slow phase. Inspirations and expirations are followed by a return of thoraco-cephalic air pressure to the atmospheric value during the forward and backward beating of the heart respectively.

03-048

MORPHOLOGY, PHYSIOLOGY, AND PEPTIDERGIC REGULATION OF THE HEART OF THE TOBACCO HAWKMOTH *MANDUCA SEXTA*.

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The heart of the tobacco hawkmoth *Manduca sexta* plays a number of critical roles during the life cycle of this organism. In addition to its obvious circulatory function, the heart is intimately involved in thermoregulation, wing inflation, and flight. In this talk I will discuss several aspects of cardiac structure and function in this species, focussing on the morphology and physiology of the adult heart and its control by neuropeptides.

03-050

THE SPATIAL ORGANIZATION OF DIGESTION IN INSECTS

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Digestive enzymes were assayed in materials derived from midgut cells and from the spaces outside and inside the peritrophic membrane (PM) from insects of different orders. Moreover, midgut fluid fluxes were studied using dyes. The data showed that in lower insects most digestion occurs inside the PM, whereas in more evolved insects only the initial stage of digestion occurs there; intermediate and final stages take place in the space outside PM or at the midgut cell surface. Most insects present a countercurrent flux of fluid outside PM which powers digestive enzyme recycling. Hemiptera insects differ from the main evolutionary stream. These insects lack a PM and their midgut cell microvilli are ensheathed by an outer lipoprotein membrane (perimicrovillar membrane, PMV). These membranes (isolated by gradient ultracentrifugation) have low density (1.087 g/cm³) and lipid content (1090 µg lipid/ mg protein), which is close to what is found in myelin sheaths. α-Glucosidase is bound to the PMV in *Dysdercus peruvianus*, and was purified to homogeneity. Immunocytochemical localization of α-glucosidase in *D. peruvianus* midgut cells showed that PMV originate from double membrane vesicles budded from differentiated Golgi areas. These results, along with digestive enzyme compartmentalization data, suggested that PMV evolved in plant sap-sucking Hemiptera ancestors as a structure to permit the absorption of amino acids present at low concentration in sap. In evolved Hemiptera displaying luminal digestion (seed- and blood-feeders), PMV compartmentalizes digestive events as PM in other insects.

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03-049

WATER CIRCULATION IN THE HONEY BEEK. Crailsheim¹, K. Visscher², G. Sherman²

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The volume of a honey bee's haemolymph can vary between 11 and 20 µl. Soon after the injection of a radioactive tracer (polyethylene glycol) into the haemolymph, the tracer can be found equally distributed within the haemolymph, indicating a rapid mixing (Crailsheim 1985, J. Insect Physiol. 31: 707-713).

Usually the crop of a honey bee contains concentrated sugar solutions. This food is transported through the proventriculus into the midgut in response to the actual metabolic need (Crailsheim 1988, J. Insect Physiol. 34: 85-90). Sugar and water are absorbed very quickly from the midgut (Crailsheim 1988, J. Insect Physiol. 34: 839-845).

We studied bees collecting water (for thermoregulation and to maintain high hive humidity for brood rearing) over long distances in a desert. Returning water foragers had crops full of water, with almost no sugar. When a water forager must fly for a long time before reentering the colony, water, instead of sugar, enters the midgut when the proventriculus opens. When this happens, bees absorb about one quarter their own weight in water, and excrete most of it within about 20 minutes. In this time they exchange a volume of water at least as large as that of their entire haemolymph.

03-051

THE MIDGUT AS A COORDINATED EPITHELIUM

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The insect midgut must respond to the ingestion of food by secretion of digestive enzymes and initiation of absorptive processes. In insects that feed continuously, there is little evidence for a co-ordinated response to the diet. However, in the intermittent feeders, in particular the haematophagous species, the cellular responses to ingestion are predictable co-ordinated both spatially and temporally. This co-ordination is also evident in the secretagogue response, where enzymes are produced according to the quality and quantity of the diet.

This presentation will provide an overview of processes in the insect midgut that can provide evidence for the insect midgut epithelium acting in a co-ordinated manner to initiate synthesis and secretion of digestive enzymes and peritrophic matrix, and absorption of dietary and digestive products. The overview will suggest mechanisms by which such co-ordination and control can occur, and highlight some areas where further research is clearly necessary.

03-052

CELL DIFFERENTIATION IN THE *MANDUCA* MIDGUT EPITHELIUMK.M. Baldwin¹, R.S. Hakim^{1,2}, M.J. Loeb² and S. Sadrud-Din^{1,2}¹Department of Anatomy, Howard University, Washington, DC USA²Insect Neurobiology and Hormone laboratory, USDA, Beltsville, MD USA

In the larval *Manduca sexta* midgut epithelium, undifferentiated stem cells proliferate prior to the molt and then differentiate to become goblet or columnar (absorptive) cells during the molt. Cell differentiation follows the pattern seen in the embryo, except that the newly differentiated cells are intercalated among mature larval cells. A distinctive cell pattern is observed in both embryos and larvae. Single goblet cells are surrounded by a one-cell-thick reticulum of 3-6 columnar cells. This pattern is observed as soon as the two cell types can be identified. Establishment of such a pattern requires some type of cell signalling. The dye Lucifer yellow will not transfer between larval midgut epithelial cells during the intermolt periods, but the cells become dye-coupled temporarily at the time the pattern is established in the newly differentiating cells. Thus, the signal for pattern formation could be passed through gap junctions. To study the effects of gap junctions on cell patterning, we have begun to isolate the gap junction protein from larval midgut epithelium. Fractions highly enriched in both gap and septate junctions are also enriched in proteins of 80 kDa and 95 kDa. Preliminary data suggest that the gap junction protein is 80 kDa. Cultures of larval midgut epithelial cells have been developed to study controls on cell proliferation and differentiation. Stem cells will proliferate in culture in the presence of 20 hydroxyecdysone and fat body extract. Stem cell differentiation in cultures only occurs in the presence of a factor produced by the mature larval cells.

03-054

CHARACTERISATION OF PERITROPHIC MEMBRANE PROTEINS FROM THE LARVAE OF *LUCILIA CUPRINA*
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The peritrophic membrane (PM) is a semi-permeable chitinous matrix lining the gut of most insects and is thought to have important roles in the maintenance of insect gut structure, the facilitation of digestion and protection from invasion by microorganisms and parasites. Proteins are integral components of this matrix although the structures and functions of these proteins have not been characterised in any detail.

The PM from the larvae of the fly *Lucilia cuprina*, the primary agent of cutaneous myiasis in sheep, was shown to contain 6 major integral PM glycoproteins, two of which together represent >70 % of the total mass of the integral PM proteins. The cDNAs coding for 3 of these proteins were cloned and sequenced. The deduced amino acid sequences indicated that each of these proteins contain 5 similar, but non-identical domains, each of ~ 70 amino acids and characterised by a specific register of 6 cysteines. One of these domains was also present in the non-catalytic regions of chitinases from *Brugia malayi*, *Manduca sexta* and *Chelonus*. There was a uniform distribution of these proteins throughout the PM. RT-PCR indicated that the PM proteins were primarily expressed in larvae and were synthesised by cells in the cardia. One of these proteins, peritrophin-44, binds reacylated chitosan and tri-N-acetyl chitotriose in vitro suggesting that these proteins bind directly to chitin fibrils within the PM. Antibodies to each of these proteins, when fed to larvae, inhibited the growth of the larvae by a common mechanism which involved the blockage of the pores in the PM and the subsequent starvation of the larvae. These results indicated that PM proteins play a key role in the maintenance of PM structure and in the determination of the porosity of the PM. Furthermore, it was demonstrated that PM proteins are effective targets for immunological control of the growth of these larvae.

03-053

DEVELOPMENT OF A POLARIZED EPITHELIUM: THE V-ATPASE AS A MEMBRANE MARKER IN THE MOULTING MIDGUT OF *MANDUCA SEXTA*.

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For our study on differentiation of epithelial cells and the biosynthesis of domain-specific membrane proteins we make use of the moulting midgut of larval *Manduca sexta*. During the moults unpolar stem cells proliferate and differentiate into mature columnar or goblet cells (Baldwin and Hakim 1991, Tissue & Cell 23, 411). The plasma membrane V-ATPase is the primary ion pump that energizes epithelial transport; it is localized only in the goblet cell apical membrane and may serve as a membrane marker. We now investigated the spatial and temporal distribution of the V-ATPase and its mRNA during moult by immunocytochemistry and *in situ* hybridization (Jaeger et al. 1996, J exp Biol, in press).

In mature goblet cells, immunoreactivity of the apical membrane is lost during moulting but re-appears shortly before ecdysis; this is due to dissociation of subunits of the peripheral V₁ sector from the membrane V₀ sector (Sumner et al. 1995, J Biol Chem 270, 5649). The mechanism of reconstitution, either by re-assembly of old or new V₁ sectors from the cytosol or by complete turnover, is not yet known. Slightly increased mRNA signal in mid-moult found for a V₁ subunit in mature goblet cells, points to enhanced biosynthesis. In differentiating goblet cells, the endosomal goblet cavity precursor compartment (GCPC) gains immunoreactivity in late moult. The V-ATPase signal of this membrane increases in intensity and area after fusion of the GCPC with the apical domain. Therefore, the V-ATPase is sorted into a membrane which changes its domain status and, thus, may not keep its target definition. Surprisingly, considerable mRNA-labelling was found in both mature cell types and in all proliferating cells from the beginning. In columnar cells, however, immunolabelling is found only transiently in the cytosol of the mature cells in apically secreted cell portions. To approach the problem of V-ATPase sorting and assembly, we currently investigate the effects of cycloheximide and brefeldin A, inhibitors of the biosynthetic pathway.

03-055

VECTOR PARASITE INTERACTIONS IN THE INSECT MIDGUT

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It has been generally assumed that the insect plays a largely passive role in the vector parasite relationship to the extent that in many cases vectors of parasitic disease are regarded only as a 'flying syringes'. It is becoming increasingly clear that insects and the parasites they transmit have a far more intimate relationship especially in the insect midgut.

In the cases of malaria and leishmania the parasites have evoked the use of an enzyme (chitinase) at different points in their life cycles to penetrate the chitinous tissue of the vector and facilitate transmission. Some insects (for example tsetse) have evolved efficient sugar dependant systems to recognise and destroy incoming parasites; parasites which evade this primary defence utilise the same host molecules as signals for their differentiation and subsequent 'exit' from the vector. In tsetse lectins secreted in the insect midgut control both establishment of the parasite infection (signalling cell death) and conversely also appear to be essential for trypanosome migration from the midgut and subsequent development to mammalian infective form (signalling maturation). These examples raise important considerations concerning the nature of the host parasite interface - it is in the parasite and vectors best interests that infections are maintained in a controlled manner and for as long as possible within the insect.

03-056

PUTATIVE ENDOCRINE CELLS IN THE OPAQUE ZONE OF *STOMOXYS CALCITRANS* (DIPTERA: MUSCIDAE)
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Cell bodies are present in the opaque zone of sugar fed *Stomoxys calcitrans* which are immunoreactive to the vertebrate opioid β -Endorphin. Cells resembling endocrine cells of the vertebrate gastrointestinal tract are present in the midgut of *Stomoxys calcitrans*. At the ultrastructural level, endocrine-like cells are seen in close association with epithelia which synthesise and release proteases in the opaque zone of *Stomoxys calcitrans*. In an attempt to understand the role of these peptide-containing cells in the digestive physiology of the insect, the intention is to investigate any correlation between changing intracellular concentrations of peptides and proteases during processing of the blood meal. This will include further double immunolabelling of β -Endorphin immunoreactive material and trypsin-like enzyme in the opaque zone and the development of a suitable competitive ELISA to measure changing concentrations of β -Endorphin immunoreactive material. Immunoreactive peptide/peptides will be further characterised by use of HPLC analytical methods.

03-058

MECHANISMS OF TRYPSIN SECRETION
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Trypsin secretion was verified in two Diptera Muscidae species using immunocytochemical techniques. The bacteria-feeding *Musca domestica* larvae do not accumulate secretory vesicles in their midgut cells and trypsin produced is fully active, unlike the blood-feeding *Stomoxys calcitrans* flies, which accumulate numerous secretory granules in a specialized midgut region (opaque zone), that are released upon stimulation, and trypsin produced is activated only when in the gut lumen. Biochemical data indicate *M. domestica* soluble trypsin is derived from an intracellular membrane form. The data indicate that *M. domestica* trypsin is secreted via an exocytic route of discharge of secretory vesicles. Inside these vesicles, trypsin was immunolocalized on the membrane. Membrane trypsin is not affected by papain or phosphatidylinositol-specific phospholipase C; is similarly solubilized by detergents with high or low critical micellar concentrations; solubilization increases at high pH values in the presence or absence of detergent. Solubilized membrane-trypsin behaves as a hydrophilic protein. The data suggest that trypsin is bound to the secretory vesicle membrane by a hydrophobic peptide anchor. Upon exocytosis, trypsin solubilization must occur because the neutral luminal pH causes a conformational change which hinders part of the trypsin anchor. Attaching trypsin to vesicle membrane may represent the insect's strategy to protect midgut cells from proteolytic damage in the absence of zymogens. Opaque zone secretory granules were heavily stained for trypsin both in unfed and blood-fed *S. calcitrans* flies. Heterogeneity in labelling intensity in different granules from a single cell and the appearance of new small and heavily stained granules, close to the apical membrane in fed flies, suggest the occurrence of preferential release of newly-synthesized granules, and possibly of non-parallel secretion. Trypsin-stained intact secretory granules and cell debris were found in the ectoperitrophic space of the opaque and lipid zones of blood-fed flies, supporting the assumption that some trypsin is released by apocrine means. Our data indicate trypsin secretion in *S. calcitrans* flies occur mainly by the classical exocytic route. The minor amount of trypsin secreted by apocrine means could represent a way of rapidly releasing enzyme soon after a blood meal is taken. Supported by: FAPESP, CNPq and British Council.

03-057

MICROVILLAR MEMBRANES FROM MIDGUT CELLS
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Although the midgut microvillar membranes seem to be important in terminal digestion, little is known about their chemical composition. By differential precipitations, we isolated microvilli from different midgut regions of larvae of *Tenebrio molitor* (Coleoptera), *Rhynchosciara americana* (lower Diptera), *Musca Domestica* (higher Diptera) and *Spodoptera frugiperda* (Lepidoptera). The microvillar preparations were treated with hyperosmotic Tris buffer, then diluted, centrifuged, and the purified microvillar membranes were recovered from the resultant pellets. Specific activities of marker enzymes in purified membranes were 1.5-3.0-fold higher than in the original microvillar preparations, except for the membranes from the anterior midgut of Lepidoptera, where no enrichment was achieved. The final yield was about 20% in all preparations. Contamination by soluble proteins was under 0.3%, and by other membranes never exceeded 5% ,as judged by chromatography in Sepharose 4B and/or by sucrose gradient ultracentrifugation. The microvillar membrane from posterior midgut of Lepidoptera was contaminated by glycogen, which can also be seen on electron micrographs. In comparison with mammals, insect membranes are rich in carbohydrates (all insects except *S. frugiperda*), cholesterol (*T. molitor*), lipids (*T. molitor*), and proteins (*M. domestica* and *S. frugiperda*). Our results suggest that the initial microvilli preparation of *S. frugiperda* anterior midgut are isolated with no cytoskeleton contaminants, needing no further purification steps. They also suggest that the amount of protein found in the microvillar membranes depends on the phylogenetic position of the insect and, along the midgut, on the amount of membrane bound enzymes or membrane bound transporters. Supported by FAPESP and CNPq.

03-059

CHEMORECEPTORS OF THE POTATO TUBER MOTH,
PHTHORIMAEA OPERCULELLA ZELL. (LEPIDOPTERA
GELECHIIDAE).

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Description and distribution of moth chemoreceptors on different body appendages by the use of optical and scanning electron microscopes, revealed present of :

1. Antenal chemoreceptors: Include sensilla trichodea (types a and b), sensilla chaetica and sensilla coeloconica.
2. Proboscis chemoreceptors: The apical region bears sensilla chaetica at its apex and rasp-like structure along its length, while the distal region bears two types of sensilla styloconica, among which are found sensilla trichodea and sensilla chaetica.
3. Tarsal chemoreceptors: Include one type of sensilla in the form of curved sensilla trichodea (type b) on the different tarsal segments and the two pulvilli that bear various sensilla including macrotrichodea, chaetica and chemoreceptor setae.
4. The ovipositor lobes: Bear mechanoreceptor and chemoreceptor sensilla, in the form of trichoid sensilla (types a and b) and 5-6 macrotrichodea.
5. The male copulatory organs: (represented by two harps and anal papillae) bear different receptors in the form of sensilla trichodea pheromonal receptors (acted in S. trichodea, type b) and pegs on the two harps, and numerous numbers of sensilla styloconica on the two anal papillae.

03-061

STRUCTURE AND DEVELOPMENT OF THE INTERNAL REPRODUCTIVE SYSTEM OF *APHELOCHEIRUS AESTIVALIS* (F.) (HETEROPTERA: APHELOCHEIRIDAE)

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The histological structures and morphological arrangement of male and female internal reproductive system of *A. aestivalis* have been studied in nymphs of instars 1-5 and variously aged apterous adults. The anlagen of gonads are well apparent in the first instar, differentiation of testicular follicles and ovarioles starts in the instar 2 and is finished in the 3rd instar. Two pairs of the accessory mesadenia are differentiated in the same way. Morphological differentiation among germ cells descendants happens during the late instar 4 and instar 5 as well as formation of the germinal trophic core from a membrane labyrinth in females. Vitellogenesis is started before imaginal moulting.

The timing of crucial reproductive processes in *A. aestivalis* seems to be rather different from the situation in most of other aquatic Heteroptera. For instance, the evidently permanent, spring to autumn gradual, multi-waved spermatogenesis and the presence of mature sperm in spermatheca of teneral, newly hatched females indicate possible semivoltinism as far as the life cycle is concerned.

03-060

MOUTHPARTS MORPHOLOGY OF A CARNIVOROUS MAYFLY, *MIRAWARA MEGALOPREPIA* RIEK (EPHEMEROPTERA: AMELETOPSIDAE)

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The morphology of fully-grown larvae of *M. megaloprepia* was studied with emphasis to its carnivorous habits. Contrary to most other mayfly larvae which are collectors, scrapers or unspecialized shredders the mouthparts of *M. megaloprepia* are highly specialized to engulfing the whole benthic animals. Labrum is large provided with stout spines on the ventral surface, covering all the remaining mouthparts; Maxilla with considerably reduced molar part and conspicuous movable teeth with membranous base; both maxillary and labial pales are uniquely multi-segmented; hypopharyngeal superlinguae strongly reduced. A lot of types of strong seatac and spines specialized for grasping the prey are described not only on mouthparts but also on the fore legs. All the mouthparts are very flexible at their bases. Epipharyngeal area can be enormously widened. Proctodcum forms a membranous retractile crop. The relationships between this mouthpart arrangement and other types occurring in mayflies are discussed with regard to ecological and phylogenetic aspects.

03-062

MORPHOLOGICAL STRUCTURES OF THE OVIPOSITOR IN THE EULOPHINAE (HYMENOPTERA: EULOPHIDAE)

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Morphology of the eulophin ovipositor is studied basing mainly on palaearctic material. Sclerites of the ovipositor (gonapophysis, gonostyl, gonocoxite), their shape, junction, apodemes and various sensillae on these sclerites are described for the Eulophinae for the first time and coeloconic sensillae were found on the sclerites also for the first time.

The research showed that different tribes of the Eulophinae have various structures and their junction. The analysis of evolutionary transformations of gonostyl and gonocoxite are presented in eulophin groundplan where gonostyl has numerous trichoid sensillae. That ovipositor is used for puncture of leaf-vein Lepidoptera. Another morphological modification is represented by a complete knit of gonostyl and gonocoxite though gonostyl has lost all the sensillae. The intermediate type of the ovipositor has membranous junction of gonostyl and gonocoxite that has only trichoid sensillae. The ovipositor of the last 2 types inserts the eggs into integument of Noctuidae larvae. These evolutionary transformations are found as an adaptation to ovipositing on active living host. Those structures can be regarded as an apomorphic state and they are important for transferring those external parasites as are eulophids to new ecological niches.

03-063

THE SUBGENUAL ORGANS OF *LOCUSTA MIGRATORIA MANILENSIS* (May.)
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The structure of the subgenual organs in the for-, mid-, and hindleg of oriental migratory locust *locusta migratoria manilensis* was investigated in detail. In each leg the subgenual organs consist of two scolopale organs: the proximal and the distal organ. In the for-, mid-, and hindleg, the scolopidia of the subgenual organs are 42 , 38 , and 31 , that of proximal subgenual organs are 28 , 24 , and 20 , and in the distal organ, they are 14 , 14 and 11 . The morphology of the trachea in the mid- and hindleg is significantly different found that found the foreleg.

The location of the subgenual organs of locust is the same with that of the tibial tympanal organs of cricket and bush cricket that have the same innervation. The shape of the proximal organs is the same with just like that of the subgenual organs of the latter. The never 5B₁ and the tympanal never (TN) have the same origination. The arrangement of the scolopidia of the distal organ can comparably with those of the intermediate organs of cricket and bush intermediate organ and the crista acustica in Tettigoniodea and Grylloidea.

03-065

PHYLOGENETIC EXAMINATION OF THE SURFACE STRUCTURE AND ARRANGEMENT IN LEPIDOPTERAN SCALE(LEPIDOPTERA)
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Almost whole body of Lepidoptera is covered with scales differentiated into various patterns ranging from sensory hairs to ordinary scales.We carried out a comparative study using SEM on the surface structure of scales of various genera classified as Lepidoptera, which are distributed widely throughout the world. The relationship among the surface structures, the arrangement and color- patterns of scales was also examined. The surface structure of scales can be classified phylogenetically into several types. The arrangement of scales is closely related with these properties of scale.The stable color-patterns might be organized. This phylogenetic classification is applicable to every species of the order Lepidoptera.The surface structures of scales appears to be common in every species of each genus. Different structures of scales between each genus,however,suggest the phylogenetic development of scale and the specific evolution of cover scales causes a wide variety of color-patterns. The arrangement of scales also varies from a random arrangement to a concentric circular arrangement around the thorax. In conclusion there are five characteristics in these morphological development of scales.
1. There is no relationship between the color-pattern and the arrangement of scales on the wing surface.
2. The hairs are randomly positioned without any regularity and do not contribute formation of the color-pattern.
3. The arrangement of scales evolves in the concentric circles direction centering on the thorax.
4. The position and the arrangement of both hairs and scales are independent of each other.
5. In the group which has either pigment projections or random projection structures on the wing surface,the development of pigment projection and random projection structure is reversely proportional to the development of scale in that portion: as the structure develop,the scale degenerate.

03-064

EVOLUTIONARY TRENDS IN TACHINID EGG MORPHOLOGY (DIPTERA, TACHINIDAE)
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I have studied the egg morphology of 112 species of 93 genera of tachinid-flies with different methods of egg-laying. In the egg structure of different groups morphological parallelisms (homoplasy) are widely spread. Within subfamilies Exoristinae (e.g. in tribes Blondelliini, Erycini), Tachininae and Phasiinae (in some groups of genera) there form eggs of transitory type, which combine features of macrotype and membranous eggs. The following transformations were noted in the egg structure of Tachinidae: 1) decrease of sizes and increase of a degree of incubation (microoviparian: Goniini); 2) increase of sizes as well as a degree of egg incubation (some Exoristinae, some Phasiinae, Voriinae, Dexiinae and Tachininae); 3) development of two aeropylar areas situated on the poles (usual) or egg sides (rare); 4) consolidation of cripts in the aeropylar zone (some Goniini, some Exoristini); 5) development of separated single aeropylar centre (some Goniini, macrooviparian Phasiinae); 6) division of the chorion into two parts: dorsal and ventral (microtype and most macrotype eggs); 7) melanization of the egg shell (Goniini); 8) there appears a devise for hatching and letting out of the 1st instar larvae: a) a lid of various structure; b) a coneshaped protuberance; c) an operculum; d) a belt of aeropylar cripts forming a seam (a-d - some Exoristinae); e) a longitudinal seams (some Phasiinae); f) a transversal seam (some Tachininae); 9) development of a plastron surface; 10) reduction of a plastron surface as well as respirative cripts (Tachininae, Dexiinae, some Exoristinae, some Phasiinae); 11) development of membranous chorion (ovolarviparian species); 12) thickening of the chorion (in eggs of microoviparian and most macrooviparian species); 13) get thin of the chorion (in eggs of most part of ovolarviparian species.

03-066

MORPHOLOGICAL ANALYSIS OF THE IMMATURE STAGES OF *SARCONESIA CHOROGASTER* (WIED.) (DIPTERA: CALLIPHORIDAE: TOXOTARSINAE)
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Sarconesia chlorogaster Wiedemann, 1830 is the only species of Toxotarsinae that occurs in Brazil, where it is limited to the southern region. This species has a high level of sinantropy and can be easily collected on decaying organic material.
From specimens reared under laboratory conditions on artificial oligidic diet, the external morphology of the egg and the immature stages (three larval instars and puparium) were described and illustrated. The cephalopharyngeal skeletons of the three larval instars were described and illustrated also.
The morphological analysis using the scanning electron microscopy showed details of unkown structures in the larvae: structure of the spines bands; presence of sensorial papillae in the oral region; presence of branched projections of the perispiracular glands; presence of a button in the base of the posterior spiracle; and in the puparium, the presence of a pair of respiratory horn.

03-067

THE IMPORTANCE OF MESOSCUTUM STRUCTURE IN TAXONOMY AND SYSTEMATICS OF THE HOVERFLIES (DIPTERA: SYRPHIDAE)

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In many recent papers which deal with taxonomic problems in the family of hoverflies one of character in use is the relief structure of mesoscutum. The authors make differences between some closely related species with type of puncturation: rough, dense, fine etc. The dusting and lustrous of mesoscutum are also often used characters.

In the analyses of mesoscutum scanning electron microscope JEOL-JSM 35 was used. Structure of sclerite surface in details is thoroughly studied in the species of three genera: *Cheilosia*, *Merodon* and *Paragus*.

Base on the results of the examination the estimation of the validity of mesoscutum characteristics used in taxonomy of the family is given. Status of couple of closely related taxa is elucidated. The differences in mesoscutum structure of springtime and summertime generations of a few species are studied. Finally, the potential significance of the examined structures in the systematics of the fly-group has been suggested.

03-068

OCCURRENCE AND ORIGIN OF PYGOPHORAL PARANDRIA IN THE PENTATOMOIDEA AND ARADOIDEA (HETEROPTERA)

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Parandria - a pair of projections of pygophore, fixed or movable, cut off by a line of descle-rotization, with no associated musculature - are developed in three taxa, viz. Discocephalinae and Podopinae from the family Pentatomidae and family Aradidae. A gradual transformation series leading to development of parandria exists within the representatives of Aradidae. Parandria are always fixed firmly to the pygophore. Two pairs occur in some aradids. The pygophore of Discocephalinae bears never movable "posterolateral projections", delimited by a sulcus from the pygophore. *Podops* generic group (8 genera), tribe Podopini (Podopinae), has developed true parandria - movable, hollow lobes, connected by a membrane with the pygophore, and covering a large area of its dorsal surface. The parandria of such form represent an autapomorphic situation within the Pentatomoidea.

03-069

COMPARATIVE MORPHOLOGICAL STUDY OF LARVAL AND PUPAL STAGES OF *HELICOVERPA ARMIGERA* (Hb.) AND *HELIOTHIS PELITIGERA* (D. ET S.) (LEPIDOPTERA NOCTUIDAE HELIOTHINIINAE)

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Studies on the morphology of *Helicoverpa armigera* (Hb.) and *Heliothis peltigera* (D. et S.) were conducted at the Tobacco Experimental Institute by means of field and laboratory observations.

The new-born larvae show slight differences at pinacula, which in *armigera* are a little bigger than in *peltigera* and have, in L1 setae, the anterior margin flattened or concave, while it is always circular in *peltigera*.

In mature larvae the differences can be summarized as follows: a) the cuticular spinules of *peltigera* are of two different kinds - big, rare, of white colour, and little, dense, of black colour - while in *armigera* they are of one kind, short, squat and very close; b) spiracles are symmetric in *armigera* and slightly asymmetric in *peltigera*; c) the cuticular spinules of the big kind can be present on the pinacula of *peltigera*, while in *armigera* they are always absent.

Slight differences were found also in the pupal stage:

a) in *armigera* the portion of maxillae protruding over the mesothoracic legs is long about half the distance between the tips of antennae, but of almost equal length in *peltigera*; b) the cremaster in *armigera* is slightly prominent, with the basal tubercles of spines always present, while in *peltigera* the prominence is missing and the tubercles are only sketched

03-070

MORPHOLOGY OF THE QUITINIZED STRUCTURES OF MUSCIDAE SPERMATHECA (INSECTA: DIPTERA)

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Little is known about the morphology of Muscidae (Diptera) spermatheca. Most papers illustrate only the capsule, which shape has little systematic significance when analyzed as a separate character. The observation of the quitinized structures of the spermatheca of some Muscidae species, made it possible to elucidate and understand characteres, which can be used in further phylogenetic analysis. 20 species of different sub-families were studied: *Biopyrellia bipuncta* (Wiedemann), 1830; *Musca domestica* Linnaeus, 1758; *Philornis univitattus* Dodge, 1968; *Charactrella malacophaga* Lopes, 1938; *Cyrtoneurina polystigma* (Wulp), 1896; *Neomuscina neosimilis* Snyder, 1949; *Brontaea debilis* (Williston), 1896; *Graphomyia mexicana* Giglio-Tos, 1893; *Mydaea plaumani* Snyder, 1941; *Scutellomusca marginata* (Albuquerque), 1954; *Dolicophaonia gallicola* (Albuquerque), 1958; *Phaonia nigriventris* (Albuquerque), 1954; *Limnophora saeva* (Wiedemann), 1830; *Coenosia camorinensis* Albuquerque, 1956; *Neodexiopsis paulistensis* Albuquerque, 1956; *Atherigona orientalis* Schiner, 1868; *Stomoxys calcitrans* (Linnaeus), 1758; *Muscina stabulans* (Fallén), 1817; *Ophyra solitaria* Albuquerque, 1958 and *Hydrotaea nicholsoni* Curran, 1939. The structures are very delicate, specially the spermathecal and expulsor ducts. No furca was observed. Two of the spermathecal ducts are fused just before entering the uterous. The following characteres and their states, were used to characterize species: 1. Shape of the capsule: round, pear-shaped or elongate; 2. Surface of the capsule: smooth to very rugose; 3. Pores at the capsule: absent or present (at base, apex or scattered); 4. Relative shape and size of the capsules: equal or different; 5. Spermathecal duct length: very short to very long; 6. Relative length of the spermathecal ducts: equal or one longer.

03-071

THE ULTRASTRUCTURE OF THE OUTER CHORIONIC SCULPTURING OF EGGS OF BRAZILIAN PHLEBOTOMINE SAND FLIES OF THE SUBGENUS *LUTZOMYIA* FRANÇA (DIPTERA: PSYCHODIDAE)

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The outer chorionic sculpturing of phlebotomine sand flies has long been noted to have interspecific variations. In the present work, chorionic sculpturing of eggs of 6 sand fly species of Brazil was examined, for the first time, with the scanning electron microscope (SEM). Three patterns could be identified. Parallel ridges were observed in only *L. whitmani*, subgenus *Nyssomyia*. Polygonal chorionic patterning was detected in *L. migonei*, *L. carmelinoi*, *L. lenti* and *L. evandroi*, all belonging to the species group *migonei*, and *L. fischeri*, belonging to the subgenus *Pintomyia*. The mountain-like pattern was observed in the chorion of eggs of *L. wellcomei*, subgenus *Psychodopygus*. Although all these species but *L. carmelinoi* and *L. lenti* can be easily differentiated by the chorionic sculpturing, it is clear that there is no unique association of a particular pattern with a taxonomic group. Further investigations are in progress on whether or not different chorionic sculpturings reflect different microclimatic conditions present in the oviposition sites.

Financial support from WHO/TDR (M8/181/4/Q.21) and NIH (AI 16305-17).

03-073

MORPHOLOGY, FINE STRUCTURE AND FUNCTION OF PROSOMAL GLANDS IN *TYPHLODROMUS* spp. (ACARI: MESOSTIGMATA: PHYTOSEIIDAE).

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On the glands of the prosomatic region in Mesostigmata there are few reports particularly in Phytoseiidae: there is infact very little information both by an anatomical point of view (Starovir, 1973; de Lillo & Aldini, 1994) and their feeding behaviour (Flechtmann & McMurtry, 1992).

A morphological and ultrastructural study on the prosomal glands in females of *Typhlodromus exilaratus* Ragusa and *T. rhenanoides* Athias-Henriot (Mesostigmata: Phytoseiidae) has been made by means of light, scanning and trasmission electron microscopy. All of the studied glands are composed of more than one cell, sometimes with a different cytological structure.

A functional interpretation of them has been proposed on the basis of their anatomy.

Our observations have revealed:

- a salivary gland complex, dorsally located in the propodosoma and connected with the salivary styli;
- coxal glands, observed latero-ventrally located at the level of the first and second pair of legs, which discharge their secretion near the coxal base of the first legs;
- a pair of glands found under the cheliceral base, above the pharynx and in front of the brain; their function is not well understood.

03-072

PROPOSED TECHNIQUES FOR DETECTION OF CHEMOSENSILLA ON THE ANTENNAE OF ADULT DIASPIDIDAE (HOMOPTERA, DIASPIDOIDEA)

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Transmission electron microscopy does not allow the study of a large number of species, mainly because of the need of live insects to process and the long time required for the preparation that must be obtained in a specialized laboratory.

The detection of chemosensilla on the antennae of adult diaspidid can also be obtained with the techniques proposed here:

- i) Confocal Laser Scanning Microscopy (CLSM) allows to observe, on balsam preserved specimens, the dendritic branch channels of the shaft of chemosensilla. CLSM offers the optical sectioning thus it shows the presence of chambers into the shaft; furthermore, CLSM improves both resolution and deep of field if compared with the traditional light-microscopy.
- ii) ii) Fluorescent microscopy can be used to label specifically the cells structures. Several fluorophores appears to be useful to observe sensillary sinus of chemosensilla.
- iii) Crystal violet staining allows researcher to detect chemosensilla; moreover, it is possible to distinguish porous from aporous regions of the shaft. This method was originally proposed by Slifer; it has been modified by Porcelli using the Karnovsky's fixative and the wetting agent TWEEN 80 and was adapted to be used during collection trips.

03-074

MORPHOLOGY AND ULTRASTRUCTURE OF ALIMENTARY CANAL IN *BACTROCERA DORSALIS* (HENDEL)

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The alimentary canal of *Bactrocera dorsalis* was studied by means of the light microscopiy and the electron miicroscopy. This alimentary canal is a long slander tract composed of the foregut, midgut and hindgut. The foregut is very small and short found in the cervical region and the anterior part of the prothorax, consisting of the oesophagus, crop, and cardia. The oesophagus appears as a tiny tube from the mouth to a bun-like shape of the cardia which is lying in the mesothorax. The crop is a pedunculate sac with a long stalk connecting to the oesophagus a short distance before the cardia. By the long stalk, the crop lies in the anterior part of the abdomen. The midgut is the longest part of the alimentary canal from the cardia to the evagination part of the malpighial tubules. The anterior part lying in the mesothorax is rather small and straight while the posterior coil portion is in the first four abdominal segment. The hindgut consists of the colon and rectum. The former is a coil tube and the latter is large formed as a cucumber shape in the female and s-shaped in males. Both of them have four rectal papillae.

The ultrastructure of the foregut and hindgut possesses a well structure of cuticular intima. The epithelium is moderately developed in the hindgut but barely evident in the foregut. They are surrounded by a single layer of the circular muscles. There is no differentiated proventriculus in *B.dorsalis*. The oesophagus extends into the inner portion of the cardia and the epithelium of the midgut starts from the outer layer of the cardia. There are three types of cells can be found in the midgut: the columnar cells, goblet cells and regenerative cells. The structures and the functions of the cells as well as the rectal papillae are invastigated and described in detailed.

03-075

MORPHOLOGICAL PECULIARITIES OF THE CHORIONIC STRUCTURE OF SOME BEETLE EGGS (COLEOPTERA, TENEBRIONIDAE, CETONIINAE)

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For the first time the chorionic structure and the different chorionic layers of the following beetles are described. The critical-point dried eggshell was broken to reveal the different chorionic layers. Surface and cross-section of the chorionic layers are examined by scanning electron microscopy (SEM).

Tenebrionidae:

- *Morica favi* LUCAS: The eggshell consists of two layers. The surface of the outer layer shows a rough and irregular relief as well as its cross-section. The inner layer is characterized by a smooth surface and an even cross-section.
- *Scaurus tristis* OLIVIER: The eggshell consists of two layers. The thin outer layer covers numerous crystals which are embedded into the outer surface of the inner layer. The inner layer shows a rough and irregular relief as well as its cross-section.
- *Pinelia crenata* F.: The eggshell consists of two layers. The outer layer is characterized by numerous pits which partly reach through this layer to the outer surface of the inner layer. The cross-section is rough and irregular. The inner layer is characterized by a smooth surface and an even cross-section.
- *Blaps alternans* BRULLÉ: The eggshell consists of two layers. The surface of the outer layer consists of white wax-like crystals. The inner layer is characterized by a smooth surface and an even cross-section.

Cetoniinae:

- *Eudicella woermanni* KRAATZ: The eggshell consists of at least ten layers. The outer layer shows wide cracks between the wax-like material. The second layer consists of pillar-like material with a roughly broken cross-section. The third layer is characterized by a smooth surface and an even cross-section. The next approximately six layers are similar to fabric with numerous fibres. The last (inner) layer seems to be solid with a rough and irregular relief.

The known chorion of many species of the Cerambycidae, Chrysomelidae, Passalidae, Staphylinidae et cetera in most cases possess micropyles visible on the exterior and interior surface of chorion as well as channels in cross-section. It is remarkable that the eggs of the above mentioned Tenebrionidae and Cetoniinae lack any micropyles and channels extending completely through chorion.

03-076

CORRESPONDING GENITAL MUSCLES IN MALE AND FEMALE MELOLONTHA MELOLONTHA (COLEOPTERA: SCARABAEIDAE) AS INDICATORS OF MORPHOLOGICAL CORRESPONDENCE IN MALE AND FEMALE GENITAL SCLERITES

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The sclerites of ectodermal genitalia are extremely different between both sexes in insects. In the cockchafer *Melolontha melolontha* (L.) males possess a spiculum ventrale and an aedeagus consisting of a phallobasis and two paramera surrounding the endophallus. In contrast, females possess only two pairs of reduced palpi vaginales and two setose fields. Despite these profound differences the genital musculature shows an extensive correspondence in the two sexes which has never been described in Pterygota. By myological comparison of both sexes considering origins, insertions, and topographic correlations of muscles the following hypotheses are inferred (KRELL 1996): 1. Aedeagus and vaginal palps are derivatives of different urites. 2. Spiculum gastrale and vaginal palps are derivatives of urite IX and are therefore morphologically corresponding structures. 3. The caudal part of Spiculum gastrale is of sternal or pleural origin. 4. The aedeagus is derived from urite X (or from intersegmental area IX/X). 5. The bursa copulatrix is derived from urite X (or from intersegmental area IX/X); bursa and aedeagus correspond morphologically. 6. The female genital tract has no sclerites corresponding to the aedeagus.

Reference

KRELL, F.-T. 1996: Die Kopulationsorgane des Maikäfers *Melolontha melolontha* (Insecta: Coleoptera: Scarabaeidae) - Ein Beitrag zur vergleichenden und funktionellen Anatomie der ektodermalen Genitalien der Coleoptera. - Stuttgarter Beiträge zur Naturkunde (B) 537.

03-078

ENDOSYMBIONT INCLUSION IN THE OOCYTES OF SILVERLEAF WHITEFLY, *BEMISIA ARGENTIFOLII* (HOMOPTERA: ALEYRODIDAE)

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This study investigates the process of inclusion of endosymbiotic organisms from adult silverleaf whitefly females, *Bemisia argentifolii* Bellows and Perring, into the developing ova. In dissected females, individual mycetocyte cells containing microorganisms were scattered singly among developing oocytes. Oocytes with mycetocytes included were first observed in females 16 h after emergence. The mean number of oocytes ≥ 0.1 mm in length per female increased through the fourth day after adult eclosion then leveled off.

The number of oocytes containing mycetocytes followed a similar pattern. Stages of mycetocyte inclusion followed a pattern based on size of the oocyte. Oocytes became associated with a single mycetocyte cell when they were a mean of length of 0.135 ± 0.003 mm (minimum of 0.11 mm). Mycetocytes were observed inside a common membrane with oocytes, at what becomes the pedicel end of the ova, when oocytes were an average of 0.147 ± 0.004 mm long. In the final stages of ovum development, the plasma of the oocyte completely surrounded the mycetocyte and the chorion was thickened.

Mycetocytes included in oocytes had a mean length of 33 ± 0.4 μ and width of 27 ± 0.5 μ . Although the females that were dissected had opportunity to oviposit, some retained several fully developed ova. Because inclusion of mycetocytes into oocytes is a continuous process, manipulations of endosymbionts before inclusion into the oocytes should be possible at any time during adult life; however, earlier treatment of an individual would likely affect a greater proportion of their offspring.

03-077

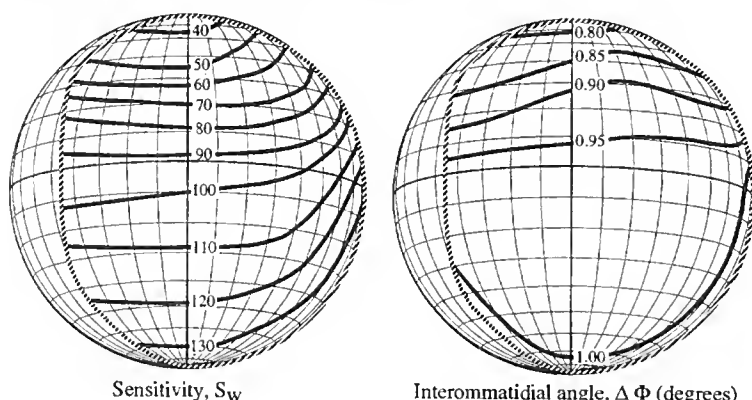
EYE DESIGN IN THE ELEPHANT HAWKMOTH, *Deilephila elpenor*

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The Elephant hawkmoth, like other moths, have an enormous field of view monitoring almost all directions except for those hidden by the body. We measured the total field of view and the visual overlap between the two eyes. We found that the overlap is largest (30° to 45°) in the antero-ventral region below the animals horizon, while above the horizon it is 30° or less. The area of largest visual overlap is where the animals see the flowers they feed on during their foraging flight.

Using optical and histological methods we obtained values for the sensitivity to white light, S_w , (Warrant & Nilsson 1995) and the interommatidial angle $\Delta \Phi$ (Snyder 1979) across the entire hawkmoth eye. We found the antero-ventral area of maximum binocular overlap to coincide with the region of highest sensitivity and, surprisingly, also the poorest resolution. At the conference, we will provide a possible explanation to this enigmatic eye-design.



Orthographic projections with the sensitivity and interommatidial angle (a measure of image grain-size) in the right eye represented by isolines. The striped border marks the part of eye where the centre of the pseudopupil or eye-glow reach the border of the eye: the maximum field of view is larger. Anterior is to the right in both figures.

References: Snyder, A.W. (1979) Physics of vision in compound eyes. In: Vision in invertebrates (Handbook of Sensory Physiology, Vol VII/6A), pp 225-313. Ed H. Autrum. Springer: Berlin. and Warrant, E.J. & Nilsson, D.-E. (1995) In prep.

03-079

MORPHOLOGY AND DEVELOPMENT OF OVARIES IN ENSIGN SCALE INSECTS (INSECTA, COCCOMORPHA: ORTHEZIIDAE)
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The paired ovaries of scale insects are telotrophic. Each ovariole is composed of tropharium and vitellarium that contains one developing oocyte. All germ cells of the ovariole originate as a result of synchronous divisions of one stem cell. The tropharia of so far investigated scale insects consist of small number of nurse cells: in Diaspididae and Coccidae - 3, in Pseudococcidae - 7. In these groups stem cells divide two or three times. In ortheziids the number of germ cells in trophic chambers is much higher (about 50 cells). Moreover, tropharium besides nurse cells contains also arrested oocytes. Both the oocyte in the vitellarium and the arrested oocytes are connected with the trophic core via nutritive cords. In the third larval instar the ovaries of *Orthezia urticae* are spindle shaped. They consist of numerous ovariole anlagen. Each anlage is composed of one germ cell cluster and has a form of a rosette. In the centre of each rosette a polyfusome occurs. During the third larval instar cystocytes differentiate into oocytes and trophocytes. Presented observations indicate that ortheziid ovarioles show some peculiar character i.e. the high number of germ cells per cluster, the occurrence of arrested oocytes and the existence of polyfusomes. Anagenesis of hemipteran ovaries is discussed in relation to the presented results.

03-081

FUNCTIONAL ANATOMY OF SALIVARY GLANDS OF *KEROPLATUS REAUMURII* *PENTOPHTHALMUS* GIGLIO-TOS LARVAE (DIPTERA: KEROPLATIDAE).

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As occur in most of the Diptera Keroplatidae, larvae of *Keroplatus reaumurii pentophthalmus* emit from the mouth a huge quantity of a mucoid, silk-like substance used, during the whole post-embrional development, for several purposes such as locomotion, protection from biotic and abiotic factors, supply of food and building of the pupal cocoon. Source of this substance are two hypertrophic tube-like salivary glands that open ventrally to the hypofarynx and extend into the abdomen, on either side of the alimentary canal. Gross-anatomy and ultrastructure of these glands were examined during the last larval stage of development. They are entirely formed by unducted unicellular secretory units that discharge the secretion into the gland lumen, which is lined with an intima surrounded by microvilli. Each cell shows a large spheroidal nucleus centrally located and characterized by polythene chromosomes. In the cytoplasm are visible typical secretory organelles such as abundant rough endoplasmic reticulum, very numerous mitochondria most varied in size and shape, free ribosomes and Golgi areas presenting a peculiar rounded vesicular appearance. Numerous secretory vesicles are uniformly distributed throughout the cytoplasm.

03-080

FINE-STRUCTURE OF THE METASTERNAL RESERVOIR OF *NEZARA VIRIDULA* L. (HETEROPTERA: PENTATOMIDAE).

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The metasternal reservoir of *Nezara viridula* is a sack-like organ ventrally located below the digestive tract and extending, with the posterior part, into the first two segments of the abdomen. It is devoted, together with the lateral glands, to the production and storage of the defensive secretion of the bug. TEM observations reveal the presence in the reservoir of two different types of secretory units: almost the whole of the surface consists, in fact, of a single layer of unicellular secretory units (type-1 SU), whereas a multilayered structure of columnar cells, constituting a peculiar strip-like organ, is limited to the ventral part of the reservoir (type-2 SU). Type-1 SU are formed by an uninterrupted layer of epithelial cells underlying the cuticular intima and separated from the haemolymph by a very thin basal lamina. Type 2 SU consists of cells presenting an intima, reduced to the epicuticular part, that forms numerous subparallel finger-like invaginations plunging in the cavity of the cells. The cytological features of the type-1 and 2 SU are described in this survey by drawings and TEM micrographs. Particular emphasis is given to the defence behaviour of the bug.

03-082

THE GENITAL ORGANS OF *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) (THYSANOPTERA: THRIPIDAE)

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The female genital organs of the thrips *Frankliniella occidentalis* consists of a pair of ovary and oviducts, a spermatheca and a single accessory gland. The spermatheca is a small-brownish orange organ adhering to the vagina, while the accessory gland is a large structure with an apical bulb and a long duct. Both organs have secretory and duct-forming cells in their epithelium. The short spermathecal duct flows into the vagina through a diverticulum of the vaginal epithelium. This also provides anchorage to muscle fibers which control the opening of duct lumen. The male genital organs consist of two testes, two deferent ducts, two seminal vesicles and two accessory glands. Seminal vesicles and glands open into a large ejaculatory bulb. The accessory glands have secretory epithelium without duct forming cells. Testes and deferent ducts are surrounded by a peritoneal sheath formed by inner muscle fibers and outer pigmented cells. The pigmented cells are not present in the accessory glands. The ejaculatory bulb continues with an ejaculatory duct, the epithelium of which is lined by a thin cuticular intima. At this level the peritoneal sheath becomes thin and has muscle fibers with intermingled pigmented cells, with the latter adhering to the epithelium.

03-083

ULTRASTRUCTURE AND DISTRIBUTION OF A FLAT-TIPPED SENSILLUM IN BAETIDAE (EPHEMEROPTERA)

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The presence of a peculiar flat-tipped sensillum in Baetidae has been demonstrated on the antennal segments of the nymphs of *Baetis rhodani*. This sensillum bears an apical pore and its slightly enlarged tip forms a flange. It is evenly distributed over the surface of the scape and pedicel, whereas forms bridges between consecutive articles of the flagellum. On the basis of morphology and location, we hypothesize that this flat-tipped sensillum functions both as mechano- and chemoreceptor.

A comparative ultrastructural investigation carried out on Italian species pertaining to the genera *Acentrella*, *Baetis*, *Centroptilum*, *Cloeon* and *Procloeon* has revealed that this flat-tipped sensillum is not a common feature of Baetidae. Indeed, it is limited to *Acentrella sinaica* and to some other species of *Baetis* other than *B. rhodani*, namely *B. alpinus*, *B. melanonyx* and *B. buceratus*. It is not found in *B. digitatus* and *B. muticus* or in the representatives of the remaining genera of Baetidae.

This kind of sensillum seems to be a specialization of the basiconic type and constitutes a microcharacter useful for both taxonomy and phylogenetic considerations.

03-085

A SCANNING ELECTRON STUDY OF THE EXTERNAL MORPHOLOGY OF PHLEBOTOMINE SAND FLY LARVAE OF THE GENUS *LUTZOMYIA* (DIPTERA: PSYCHODIDAE) OF A CUTANEOUS LEISHMANIASIS FOCUS IN CEARÁ STATE, BRAZIL.F. A. C. Pessoa¹, R. G. de Queiroz¹, R. D. Ward²¹ Universidade Federal do Ceará, Brazil; ² Keele University, U.K.

Cutaneous leishmaniasis is a disease caused by protozoans of the genus *Leishmania*, which are transmitted to man by phlebotomine sand flies. Although the biology and morphology of adult sand flies have been extensively studied, the immature stages of these insects are not well known. As a result, sand fly species have been routinely identified by only adult characters. The present work describes, for the first time, the ultrastructure of the 4th instar of larvae of 7 species, namely *L. longipalpis*, *L. whitmani*, *L. intermedia*, *L. migonei*, *L. lenti*, *L. evandroi*, and *L. sericea*, and the taxonomic importance of external structures is assessed. Laboratory-reared larvae, first killed in hot water, were fixed in 3% glutaraldehyde, then washed thoroughly in several changes of phosphate-buffered saline, submitted in 1% osmium tetroxide for an hour and dehydrated through a series of alcoholic solutions, critical point dried, and gold sputtered. The specimens were examined with a Hitachi scanning electron microscope S 4500. Interspecific variations were observed in the arrangement of setae of the segments, and morphology of antennae, spiracles, caudal setae and mouthparts. Therefore, these characters may be used as an alternative means of identification of these species.

03-084

OVARIOLE ULTRASTRUCTURE IN *SILO MEDITERRANEUS SATURNIAE* MORETTI (TRICHOPTERA: GOERIDAE)M.C. Bicchieri¹, E. Gaino

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The organization of the meroistic polytrophic ovarioles of *Silo mediterraneus saturniae* Moretti was examined by light microscopy and both scanning (SEM) and transmission (TEM) electron microscopy.

Each ovariole is composed of a linear sequence of follicles the maturation of which is detectable by both dimension values and a superficial polygonal pattern. Structural details of egg cells, follicle cells and nurse cells were observed in follicles in previtellogenic and vitellogenic stages. The loose, net-like texture of the stalk cells is particularly evident between adjacent, fully developed follicles.

The onset of the vitelline envelope is marked by precursor material synthesized by the follicle cells in the form of electron-dense granules. The granules are extruded in the space between the membrane infoldings occurring close to the egg border. The aligned granules fuse together to form linear arrays which give the vitelline envelope an initial striated aspect. By contrast, during choriogenesis precursors are accumulated along the basal region of the follicle cells in a discontinuous layer which gradually becomes an electron-dense, thin and homogeneous sheath.

As choriogenesis proceeds, follicle cells tend to detach from the egg surface giving rise to a scallop-like layer. This arrangement is consistent with the irregularly folded appearance of the chorion in newly ovulated eggs. Under SEM, the egg inclusions are visible underneath the chorion owing to the thinness of this envelope.

The simple organization of the chorion seems to be related to the egg deposition in water-absorbing spumaline which has a protective function.

03-086

A NEW MORPHOLOGICAL STRUCTURE IN *KERMES BOTTARD* (HOMOPTERA COCCOIDEA KERMESIDAE)

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Kermes vermilio (Planchon) is a common scale insect living on *Quercus* spp. in the Southern Italy urban environment.

Morphological studies on female and male young instars have showed the presence of ventral frontal lobes. They are situated just antero-medially to each basal antennal segment and have membranous appearance.

Until now, similar lobes were known only in many species of the family Eriococcidae. These structures are here recorded in Kermesidae for the first time and probably they are present in other species of the family.

Their function is unknown.

03-087

CYTO- AND HISTOCHEMICAL STUDIES OF THE OVARY IN *PANORPA COMMUNIS* (MECOPTERA).

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The polytrophic ovarioles of *Panorpa communis* are differentiated into a terminal filament, germarium and vitellarium. The germarium contains germ cells clusters (cystoblasts and cystocytes) at early stages of their differentiation. All cystocytes enter the prophase of meiosis, so their ultimate fate can not be predicted at this stage. At the base of germarium the cystocytes become morphologically diversified. One of the central cells of the cluster become the oocyte while the remaining cystocytes transdifferentiate into nurse cells. The oocyte nucleus is spherical and transparent while the nurse cells nuclei become polyploid, they grow rapidly and are filled with numerous DNA- positive granules. The presence of intercellular bridges was revealed by rhodamine - phalloidin staining of F-actin. The intercellular bridges between the oocyte and the nurse cells were found to contain more F-actin than those connecting the nurse cells. The derivatives of one cystoblast - three nurse cells and oocyte are enveloped by follicular epithelium, thus forming an egg chamber. The follicular epithelium is morphologically diversified into a group of densely packed follicle cells around the oocyte, a highly flattened group of cells which encompass the nurse cells and a group of cells which migrate in between the oocyte and nurse cells at the beginning of vitellogenesis. This diversification is also reflected in the organization of microfilaments.

03-089

MORPHOLOGICAL ASPECTS OF ENDOCYTOBIOSIS IN COCKROACHES

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Intracellular symbiotic bacteria have been observed in all cockroaches (Blattaria) examined. They inhabit specialised cells of the fat body, are transovarially inherited, and appear to be essential for the growth and reproduction of their hosts. Molecular phylogenetic analyses placed the symbiotic bacteria of the Blattaria among the favobacteria and indicated that the infection occurred in an ancestor common to cockroaches and termites. The endocytobionts are enveloped by a vacuolar membrane derived from the host cell (the bacteriocyte). Ultrastructural studies of the host and symbiont membrane systems were performed in three Blattaria families (Blattellidae, Blaberidae, Cryptocercidae). The results show the presence of vesicles originated from blebbing processes of the vacuolar membrane and of close adhesion sites between this membrane and the prokaryote cell envelope. Freeze-fracture replicas evidence aggregates of intramembrane particles on the vacuolar membrane. A blebbing of the symbiont outer membrane is described in the Cryptocercidae. The vesicles detaching from the symbiont outer membrane could be a mechanism for releasing symbiont products in the vacuolar space. The transovarially transmitted symbiotic population is integrated in the ontogenetic processes. In *Periplaneta americana* (Blattellidae), after the acquisition of the bacteria, the formation of a "mycetome" is observed. The mycetome is made up of a syncytial envelope, probably formed by the vitellophages, that contains a considerable symbiont ball. During the embryonic development, there is a progressive decline in the bacterial population, caused by lytic processes which take place inside the mycetome. In the following phases of embryogenesis, a residual bacterial population abandons the yolk sac and moves to the epineural sinus, where it is phagocytized by the embryonic plasmatocytes which then become bacteriocytes. The formation of these cells follows a pattern similar to that observed in *B. germanica* (Blattellidae). Therefore, the bacteriocyte formation took place, within the order Blattaria, following similar coadaptive processes.

03-088

INTERNAL REPRODUCTIVE ORGANS IN ADULT MALE *POLISTES DOMINULUS* (CHRIST) (HYMENOPTERA: VESPIDAE)

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In the genus *Polistes*, as in most Hymenoptera, spermatogenesis ceases in the late pupal stage (Machida, 1934; Pardi, 1947). Apart from that, very little is known about the male internal reproductive organs. This study provides a morphological and histological description of the internal reproductive organs of male *Polistes dominulus*.

Adult males, emerged from colonies reared in the laboratory, were divided according to age, fixed in Pamp's fluid and dissected in alcohol. The reproductive tracts of some individuals were drawn under a camera-lucida and used for measurement, while others were embedded in polystyrene, sliced into 8 µm sections and stained for light microscopy.

The internal reproductive organs of male *P. dominulus* consist of a pair of testes joined medially, paired seminal vesicles, accessory glands and ducts. At emergence, the testes are loaded with sperm, the seminal vesicles show a corrugated internal surface with very few sperm, the accessory glands have a thick wall with active columnar cells and scanty secretory product in their lumina. After emergence the testes slowly degenerate and the sperm descend into the seminal vesicles. The thickness of the gland wall decreases and a lot of secretory product is found in the gland lumen. With increasing age, the testes size decreases and the gland size increases.

Males older than 2-3 days store many sperm in their seminal vesicles and are therefore capable of mating repeatedly.

The increase in size and content of the accessory glands suggests that their secretion not only contributes to the seminal liquid, but could also be functionally involved in the territorial mating system of this species. In this reproductive strategy males are active in territories for more than one month (Beani and Turillazzi, 1988).

03-090

SPIRACULAR MORPHOLOGY OF IMMATURE STAGES OF PHLEBOTOMINE SANDFLY (DIPTERA, PSYCHODIDAE).

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Insect larval spiracular system assumes a great variety of forms. Many of the changes observed in different systems are clearly adaptative. In the present investigation, the morphology of the spiracles of fourth instar larvae of eight sandfly species (*Phlebotomus perniciosus* Newstead, *P. papatasi* Scopoli, *P. perfiliewi* Parrot, *Lutzomyia longipalpis* (Lutz & Neiva), *L. ovallesi* Ortiz, *L. youngi* Feliciangeli & Murillo, *L. evansi* Nuñez-Tovar, *L. trinidadensis* Newstead were examined by scanning electron microscopy.

Larvae of phlebotomines are amphipneustic, having 2 pairs of spiracles: the metathoracic one is situated at the anterior edge of the second thoracic segment; the post abdominal pair is on the posterior corner of the eighth abdominal segment. In the larvae of the all eight species both the anterior and posterior spiracles are placed at the top of a globular body. Their structure includes an external opening with a closing apparatus and a peritreme which surrounds it. The latter has rose-like shape, consisting of a circularly arranged petal-like protuberances, separated each from the other by elongated septa. Each petal is longitudinally crossed by an elevated linea divide the petal in two identical parts.

The taxonomic and phylogenetic value the specific features of spiracular morphology is discussed.

03-091

MORPHOLOGY AND ULTRASTRUCTURE OF THE CEPHALIC VESICLES OF THE DRYININAE AND GONATOPODINAE LARVAE (HYMENOPTERA, DRYINIDAE).

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Dryinidae are parasitoids of Homoptera Auchenorrhyncha. The larvae are usually partly ectoparasitic. The posterior part of the parasitoid body protrudes from the host between two overlapping sclerites, whereas the anterior part draws in the haemocoel of the host. The head of larvae of Dryininae and Gonatopodinae shows two vesicles in front of their face. These structures are usually considered to be involved in the feeding of the first larval instars. In order to obtain new data useful in clarifying the real function of these structures, the external morphology and the internal organization of the cephalic vesicles have been examined in the following species: *Dryinus collaris* (L.) (Dryininae); *Gonatopus communis* Olmi, *G. atlanticus* Olmi and *G. camelinus* Kieffer (Gonatopodinae).

03-092

SULFATION AS A POSSIBLE MECHANISM TO REGULATE VITELLOGENIN TRANSIT THROUGH EPITHELIAL CELLS IN *CARAUSIUS MOROSUS* OVARIAN FOLLICLESA. Cecchetti¹, A. Falleni, M.T. Locci, F. Giorgi
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During vitellogenesis the fat body synthesizes a number of non-vitellogenic proteins that are transferred to the ovarian follicles along with vitellogenins (Vgs). While Vgs are stored in the oocyte to sustain embryonic development, non-vitellogenic proteins are thought to play a major role to regulate endocytic uptake and/or trigger onset of early development. A non-vitellogenic protein has recently been identified in the stick insect *Carausius morosus* (Giorgi et al., 1995). This is a fat body derived protein of 157 Kd that is selectively sulfated during vitellogenesis. Data proved that it is neither labelled with ³H-acetylglucosamine nor with ³⁵S-SO₄²⁻ in cultured fat body. This suggests that sulfoconjugation takes place upon transfer to the ovarian follicle rather than upon secretion from the fat body and that it is not associated with the carbohydrate moiety of the protein. In order to verify this possibility a number of developmentally different ovarian follicles were exposed both *in vivo* and *in vitro* to ³H-acetylglucosamine or ³⁵S-SO₄²⁻ for time exposures ranging from 30 minutes to 8 hours. Samples were then processed for fluorography, scintillation counting and high resolution autoradiography. Autoradiographs were quantitatively analyzed according to the procedure set up by Salpeter (1978). When observed at the EM level, follicle cells appear characterized by an extensive endocytic activity along the apical end and by numerous secretory vesicles throughout the cortical cytoplasm. Autoradiographic analysis of ovarian follicles exposed to ³⁵S-SO₄²⁻ demonstrate that radioactivity is first associated with the Golgi apparatus of the follicle cells, followed by secretion into the interfollicular spaces and finally it is localized over the microvillar area between the follicle cells and the oocyte. With longer time exposures to the radioisotope, some of the radioactivity appears associated with the cortical ooplasm. Data are interpreted as suggesting that the 157 Kd protein is sulfated within the follicular epithelium, following a transcytotic pathway through the follicle cells. By doing so this sulfated protein may contribute to forming the extracellular matrix of the interfollicular cell channels. In addition, it may also provide some binding sites for the incoming Vg so as to retain it in the interfollicular channels prior to interaction with the oolemma.

03-093

HEMOPOIESIS IN THE STICK INSECT *CARAUSIUS MOROSUS*M.T. Locci, A. Cecchetti¹, M. Masetti¹, F. Giorgi
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The problem concerning the origin of hemocytes in insects is far from being elucidated. While hemocytes in the adult are known to be produced by specialized hemocytopoietic organs of mesodermal origin (Ratcliffe et al., 1985), those in the embryo are thought to originate from median mesodermal cells (Romoser and Stoffolano, 1994). In many insect species, these organs have been identified and histologically characterized (Gupta, 1985). The hemocytes of the stick insect *Carausius morosus*, i.e. plasmatocytes, granulocytes and spherulocytes have already been identified in the adult (Locci et al., 1995). Cells with similar ultrastructural characteristics were also identified amongst those released by *in vitro* cultured embryonic yolk sac. In the present study, the heterogeneous cell population released by the yolk sac has been characterized both morphologically and immunohistochemically. Hemocytes in *C. morosus* were shown to react against ConA and an anti-vitellogenin monoclonal antibody (Mab B12), besides responding to a phenoloxidase test. These same reactivities were thus used to verify whether some of the cells released by the yolk sac were embryogenetically related to hemocytes. Some of these cells reacted with all three substrates, suggesting that they could potentially behave as hemocytes. Based on these observations we propose that the yolk sac may provide stem cells capable of differentiating into hemocytes upon migrating to an embryonic hemocytopoietic organ, the embryonic dorsal vessel. Several lines of evidence suggest the dorsal vessel of the adult insect as a potential hemocytopoietic organ. Dorsal vessels from a number of stick insects were thus cultured for over 15 days in Grace's medium supplemented with adult hemolymph. During this culture period different kind of cells were released into the culture medium. Ultrastructural analysis of these cells demonstrated that they are morphologically similar to different classes of hemocytes, i.e. plasmatocytes, granulocytes and spherulocytes. On the basis of these observations we postulate that the dorsal vessel in stick insects behaves as a hemocytopoietic tissue both in the embryo and in the adult. The cells that are released by the yolk sac upon *in vitro* culturing are interpreted as stem cells, partly differentiating as hemocytes and partly contributing to the embryonic dorsal vessel.

03-094

HAMANN'S ORGANS AND THEIR SIGNIFICANCE FOR STAPHYLINOIDEA BEETLES OF AGYRTID — COLONID GROUP OF FAMILIES (AGYRTIDAE, LEIODIDAE, COLONIDAE) PHYLOGENY UNDERSTANDING

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Hamann's organs are to be found on the 7th, 9th and 10th antenna segments of leiodid beetles and consist of periarticular gutters on the distal surface of 7th, 9th and 10th antennal segments and usually vestibulums and vesiculums, which often occupy the main part of the volume of the 7th and part of the 9th and 10th segments (Accordi, Sbordoni, 1978).

The 7th antenna segment of the Cretaceous form *Nyujwa* Perkovsky (Leiodidae, Camiarinae, Neopeltopini) from Baikal region is extended enormously, the 8th is strongly reduced, the 9th is slightly narrower than 7th, 10th is strongly narrower than 9th, 11th is strongly narrower than 10th.

The reduction degree of *Nyujwa* the 8th segment is common to recent Leiodidae with typical for the family interrupted 5-segmented club. A strong extension of the 7th, 9th and 10th antennal segments in recent Leiodidae is always connected with Hamann's organs development on these segments.

Easier access to the 7th segment Hamann's organs of Cretaceous Leiodidae was to be reached through the 8th segment reduction. At the same time periarticular gutter does not appear on the 7th segment distal surface of the colonid antenna, and their 8th segment is never reduced, which is proof to attribute Colonidae to the related Agyrtidae, but different from Leiodidae phyletic line.

Accordi, F., Sbordoni V., 1978. The fine structure of Hamann's organs in *Leptodirus hohenwarti*, a highly specialized cave Bathysciinae (Coleoptera Catopidae). Int. J. Speleol. 9 : 153-165.

03-095

DESCRIPTION OF IMMATURE STAGES AND LIFE HISTORY OF OXYNA PARIETINA L. /DIPTERA: TEPHRITIDAE/ ON ARTEMISIA VULGARIS /ASTERACEAE/ IN CZECH REPUBLIC

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Oxyna parietina L. is univoltine and nearly monophagous on native mugwort *Artemisia vulgaris* /Asteraceae/ in Czech Republic.
Larvae develop singly in and feed in stems of the host plant.
The egg is described and illustrated in detail in plant tissues for the first time.
The third instar larvae and puparium are described and figured using scanning electron microscopy.
Life history of *Oxyna parietina* is described and potential use as a biocontrol agent for *Artemisia vulgaris* in Czech Republic is discussed.

03-097

LTSEM MORPHOLOGICAL STUDIES ON *DICYPHUS* (D.) *ERRANS* (WOLFF) (HETEROPTERA: MIRIDAE).
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Dicyphus errans is a Mirid predator of glasshouse and field crop phytophages (mites, aphids, whiteflies) of cultivated Solanaceae and Cucurbitaceae.
Morphological observations of frozen-hydrated specimens of adults and preimaginal stages were conducted by Low Temperature SEM.
Egg characteristically show two short respiratory horns on the apex, arising from the anterior margin of the body of the shell, laterally from the elliptic operculum. The eggs are deeply embedded in plant tissue while the operculum-horn complex emerges from the leaf surface. Subsequently during embryonal development the operculum moves away from the respiratory horns, which remain attached to the chorion.
The first three antenna articles bear sensillar fields of 10-30 "smooth peg" sensilla on the dorsal surface. No differences exist between male and female adults while in the preimaginal stages sensilla reside only in first two segments. In the dorsal first article of adults and preimaginal stages there is a placoid sensilla. Numerous sensilla tricoidea and chaetia are present in all the articles. First and second instars show very numerous small smooth sensilla on the second and third articles between scattered long grooved sensilla chaetia.
Examination of the mouthpart morphology show that the rostrum has numerous "hair-like" mechanoreceptor sensilla all along its surface. The bipartite apex (separated by the dorsal stilet groove) reveals one terminal field of 9-11 peg sensilla on each lateral lobe.
Between the meso- and metathoracic adult legs, on the lower border of the episternum, there is the pterotoracic scent gland orifice. It has a peritreme surface constituted by specialized mycoid microsculpture involved in the of discharged secretion dispersion mechanism. In nymphs, on the other hand, the scent gland system is dorsal and abdominal.

03-096

ANTENNAL CHEMOSENSILLA IN *CARAUSIUS MOROSUS* BRÜNNER (PHASMIDAE)
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To date few morphological and ultrastructural observations concerning the antennal chemosensilla in stick insects (*Phasmidae*) are available. The objective of the current study is to provide further information on these sense organs in *Carausius morosus*.
Throughout the parthenogenetic female antenna there is a low number of sensilla in the first half and a markedly increasing density in the second half. Mechanosensitive sensilla of different types (peg-like, bristle-like and campaniform), a small number of poreless thermo-hygrosensitive (Altner et al., 1978) and chemosensilla have been found.
Three types of chemosensilla have been identified. Type 1 has a cuticular apparatus (outstanding hair-shaft) 100-150 µm long, gradually tapering from the base (5-6 µm in diameter) to the tip, with fluted aporous walls and spatulate tip. The cross sections of hair show a double lumen formed by the cuticle. Inside the innervated lumen there are arc unbranched dendrites of five receptor cells. They have different diameters and the pattern of their microtubules is 5x2+1 or 7x2+3. The section in the ciliary sinus shows the thick dendritic sheath that wraps the five outer dendritic segments and the tubular body. This structure is typical of sensilla with gustative function.
Type 2 is a "hair-like" sensilla. It is shorter than type 1 (80-90 µm) and has a smaller diameter (4-5 µm at the base); it has a blunt tipped and smooth walls. Ultrastructural investigation demonstrated the presence of pores and spoke channels that perforate the thick cuticular wall of the hair. This type seems to be an olfactory sensilla. The number of sensory cells is from four to six.
"Peg-like" sensilla (type 3) are less abundant and smaller (15-25 µm in length and 2-3 µm in diameter at the base), peg-shaped and blunt tipped. The hair, in cross section, shows a relatively thin cuticular wall and displays a high density of pores each leading into numerous pore tubules. The latter are surrounded by a wider liquor channel filled with receptorlymph, similar to the hair lumen. The cellular components consist of a greater and variable (5 - 22) number of sensory neurons. The dendritic segments are first enclosed in a common sheath and later divide into numerous branches.
Altner H., Tichy H., Altner I. (1978) - Cell Tiss. Res., 191: 287-304.

03-098

MORPHOLOGY OF THE STOMODEUM IN LARVAE OF SOME TRICHOPTERAN FAMILIES
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The larvae of some families belonging to the Trichoptera Order, have some cuticular structures such as spines, teeth and bristles in the stomodeum and particularly in the proventriculus.
In this study, the Authors examined, under scanning electron microscope, mature larvae from the following families and genera: Rhyacophilidae (*Rhyacophila*), Hydropsychidae (*Hydropsyche*, *Cheumatopsyche*), Polycentropodidae (*Polycentropus*, *Plectrocnemia*), Phryganeidae (*Agrypnia*), Odontoceridae (*Odontocerus*) which have a largely carnivorous feeding regime.
In *Hydropsyche* and *Cheumatopsyche*, these structures are very complicated and consist of elaborate tooth-like, spiniform processes called "gastric mills" by Boon (1983-85).
In *Rhyacophila* the spines are distributed along longitudinal ridges.
In *Polycentropus* and *Plectrocnemia* both spines and fringes of long bristles are present.
In *Agrypnia* the spines are numerous and uniformly distributed all over the proventriculus; in the crop there are a few small spine-like tubercles, and at pharyngeal level there are spines again.
Odontocerus has small combs with teeth of different sizes, distributed longitudinally at the beginning of the proventriculus and variously disposed in its caudal portion.
In conclusion, morphological examination of the stomodeum reveals differences between the various families but the complexity of the proventriculus does not seem to be related to the feeding regime. *Rhyacophila*, exclusively predatory and carnivorous, has only some series of spines whereas *Hydropsyche*, which is filter-feeder and has an omnivorous feeding regime, has "gastric mills".

03-99

HEMOLYMPH PUMPING MECHANISM IN THE
LOCUST MIDLEG

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An oblique diaphragm separates the trochanter and the femur in locust midlegs and blocks the passage of hemolymph in the leg's ventral compartment. The only distal outlet is into the ventral blood vessel of the femur. It is supplied with a flap-like valve that opens when the diaphragm is pulled proximally by two muscle bundles. This is a rhythmic movement that is usually synchronized with increased hemolymph pressure arising from the expiratory phase of ventilation. When the muscle bundles relax during inspiration the diaphragm flattens due to the antagonistic tension produced by its elastic tissue and by cross striated muscle fibres which are arranged radially on the diaphragm.

The muscle bundles that pull at the diaphragm are supplied by a single excitatory motoneuron. A different single neuron extends over the diaphragm and may have modulatory function on its muscle fibres and possibly on other parts of the leg, for immuno-histochemistry has shown a high content of serotonin in it.

03-101

THE CYTOSKELETON IN OVARIAN CLUSTERS OF
POLYTROPHIC MEROISTIC INSECTS

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We comparatively investigate the cytoskeleton of polytrophic meroistic ovaries in several insect orders (adephage coleopterans, neuropterans, hymenopterans, trichopterans, lepidopterans, mecopterans and dipterans). Well known are the dynamics of the *Drosophila* egg chamber cytoskeleton (e.g. Cooley and Theurkauf (1994), *Science* 260: 590-596). To what extent are these results also valid in other insect orders?

Our data clearly demonstrate that the occurrence of actin filaments in prominent cytoskeletal structures, like the fusome or the ovarian ring canal is no common feature typical of all orders, but differs depending on the group. For example there is no actin detectable in the *Drosophila* fusome, while in hymenopterans and especially in adephage coleopterans it seems to be an abundant component of this organelle. The association of actin with ring canals does not show a unique pattern as well, but varies in several ways throughout the groups studied so far. While the protein is largely absent to ring canals in adephage coleopterans, lepidopterans, trichopterans and mecopterans, its association with hymenopteran ring canals is restricted to a short period of cluster development.

Inquiry of additional cytoskeletal molecules during insect oogenesis is in progress.

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03-100

ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL STUDY OF
KARYOSPHERE AND NUCLEAR BODIES IN INSECTS WITH
DIFFERENT TYPES OF OVARIOLES.D.S. Bogolyubov, O.A. Alexandrova, A.G. Tsvetkov, E.M. Antipanova,
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Russia

The morphological features of diplotene oocyte nuclei were studied for some insects with different types of ovarioles: *Tenebrio molitor*, *Tentyria nomas taurica* (Coleoptera), *Chrysopa perla* (Neuroptera) and *Galleria melonella* (Lepidoptera). The ultrastructural analysis of karyosphere in different insects showed several patterns of its formation. In all species studied except *T. molitor* the karyosphere has a fibrous capsule. Apart from the karyosphere, the numerous extrachromosomal nuclear bodies (NB) of different morphological types appear in oocytes of all species studied. They seem to be involved in karyosphere capsule formation. In *T. molitor* where the capsule is absent, some NB are present in the karyosphere. According to immunocytochemical data and in situ hybridization of U2 snRNA some NB contain snRNP and non-snRNP splicing factor SC 35 and can be referred as analogues of B snurposomes of amphibian oocyte nuclei. Other NB contain the coilin-related protein and snRNP and they are similar to coiled bodies of somatic cell nuclei. In all cases the karyosphere contains snRNP. Staining of tenebrionid oocyte nuclei with antibodies against fibrillarin and in situ hybridization to rDNA gave negative results, which allows conclusion to be made that nucleoli are absent in oocytes of tenebrionids and that is why the karyosphere does not contain fibrillarin. By contrast, in *C. perla* and *G. melonella* oocyte nuclei the nucleoli exist. The karyosphere capsule in *C. perla* oocytes contains fibrillarin. It can be connected with the nucleoli involvement in capsule formation.

03-102

SPHERES (BINNEKÖRPER) FROM CRICKET AND DAMSELFLY
OOCYTES CONTAIN FACTORS OF SPLICING pre-mRNA AND
PROCESSING OF pre-rRNAA.G. Tsvetkov, M.N. Gruzova¹, J. Gall²Laboratory of Cell Morphology, Institute of Cytology RAS, St. Petersburg,
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By the method of immunocytochemistry it has been shown that spheres from cricket and damselfly oocyte nuclei contain small nuclear RNA (snRNA) and fibrillarin - protein involved in pre-rRNA processing. Besides in cricket oocyte spheres coilin-related protein has been revealed. It is known that coilin is a part of intranuclear structure called coiled body in somatic cells. By the method of hybridization nucleic acids in situ U1, U2 and U6 snRNAs has been identified in spheres of cricket oocytes. Concentration of U2 snRNA was much higher compared to U1 and U6 snRNA. After longtime (24h) incubation of cricket's ovary in medium containing ³H-uridine spheres remained unlabelled by contrast with heavily labelled karyoplasm.

The data obtained show homology between spheres from studied insects and coiled bodies of somatic cell nuclei, spheres from amphibian oocyte nuclei and prenucleolar bodies in nuclei formed in vitro in *Xenopus laevis* egg extract after adding DNA.

03-103

NOTE ON THE FEMALE REPRODUCTIVE SYSTEM OF SOME PUPIPARA (DIPTERA, NYCTERIBIIDAE, HIPPOBOSCIDAE)

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The position of Nycteribiidae in the system of Diptera is still questionable. Deep changes of their external and internal morphology caused by parasitism make it difficult to determine the relationships of these flies. Nycteribiidae possess adenotrophic viviparity, and their larvae develop in the female organism feeding on the secretion of accessory glands. These glands are great in size, tubular and very branched organs connected by the ducts with uterus-like dispair oviduct. The comparative anatomic analysis of the glands showed many similar features in their morphology in Nycteribiidae and Hippoboscidae. Symbiotic bacteria are found in all examined species. Though the secretion in both families takes place on merocrine pattern, some differences are discovered in the ultrastructure of secretory cells. Hippoboscidae have an extra-cellular reservoir at each secretory cell. The reservoir is separated from the gland duct by cuticular net. Nycteribiid cells have not such structure, and the secretion take place directly in the gland ducts. Apparently, these physiological and morphological distinctions prove phylogenetic separation of Nycteribiidae and Hippoboscidae.

03-105

OBSERVATIONS ON MORPHOLOGY AND BIOLOGY OF *CALAMONCOSIS APRICA* MG. (DIPTERA, CHLOROPIDAE)

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All the development stages of *C. aprica* were being observed from May to August in the years 1973-1974; 1985-1986. The material was collected by means of the observation, analysis of reed stems, laboratory breeding and the scop method. Morphology of the egg, the first, second, third stages of the larva and of pupa was described and illustrated. Female and male terminalia, not known so far, were described. On the bases of a few years' observations it has been established that *C. aprica* appears in great numbers in galls of *Platycephala planifrons* (F.) on Common reed (*Phragmites australis* Trin.) stems. In a particular stem there were from 8 to 58 larvae. Eggs, the first and second larval stage were found in the first half of June. The third stage was observed from the middle of June till the middle of July. Pupa appeared in the second half of June and was presente there till the middle of July. Adult were collected over the reed from the third decade of June to the third decade of August. It is supposed that *C. aprica* has two generations within one year. It is known that the Summer generation develops on the reed in galls of *Platycephala planifrons* and its larvae are phytophages. Winter generations probably appear in galls of *Lipara*, and their larvae feed rotten plant substance.

03-104

A SCANNING ELECTRON MICROSCOPICAL STUDY OF LARVAL STRUCTURES IN AFRICAN DIPTERA.

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The head structure, spines and papillae of the segments, and the anterior and posterior spiracles of larval stages were studied by Scanning Electron Microscopy.

The main morphological features are illustrated by micrographs.

03-106

ULTRASTRUCTURE OF THE ENDOCRINE CELLS IN THE MIDGUT EPITHELIUM OF THE COCKROACH, *PERIPLANETA JAPONICA*

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The endocrine cells in the midgut epithelium of the cockroach, *Periplaneta japonica* were observed by the light and electron microscope. The midgut epithelium of the last instar larva and adult cockroach was consisted of principal columnar cells, regenerative cells, and endocrine cells. Midgut endocrine cells were positioned basally in the epithelium as cone-shaped single cell or underneath the regenerative crypt cells. When midgut epithelium grows and the cell composing it transform, between the endocrine and regenerative cells were made desmosome type junction and large vesicular shaped stretches of loose contact. The endocrine cells were characterized by a clear cytoplasm with abundant Golgi complex and numerous secretory granules. The secretory granules in the cell were spherical, unit membrane bound, and osmiophilic electron dense granules about 200~400nm diameter. The secretory granules have been observed as discharged by exocytosis on the basal, lateral side of the cell.

03-107

THE FINE STRUCTURAL ASPECTS OF COCOON SILK PRODUCTION IN THE SPIDER, *ARGIOPE AURANTIA*
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The silk glands of araneid spiders are of several types. Among these, the principal fibers used in constructing the eggcase are products of the cylindrical glands (or tubuliform glands) which are present only in females. And development of these glands parallels maturation of the ovaries.

The cylindrical gland spigots of the garden spider, *Argiope aurantia*, have a noticeably wider aperture than those serving other types of silk glands, reflecting the relatively large size of the cylindrical gland fiber. Examinations of formed fibers indicate a multicomponent internal structure, and highly magnified electron micrographs reveal each fiber contains numerous electron lucent fibrils embedded in an amorphous electron dense matrix.

These heterogeneous fibers not necessarily taking place in a uniform manner throughout the length of the cylindrical gland. By the several evidences obtained from light and electron microscopical observations, a mechanism related to eggcase silk formation can be presented.

03-109

THE EGG SHELL FORMATION AND CHORION FINE STRUCTURE OF *HYDROMETRA STAGNORUM* (HETEROPTERA)
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The hydrometrid egg shell layers are secreted by follicular epithelium, after the vitellogenesis has been completed. The vitelline membrane is the first to be formed, and its formation is preceded by an increased of rough endoplasmic reticulum and increase in the number of dictyosomes, then by accumulation of secretory vacuoles which are secreted from the cell to the space between the oocyte and follicular cells. There the material accumulates, fuses and forms consecutive layers on the surface of oocyte. The vitelline membrane is formed around the entire oocyte and shows no local modifications. The chorion is two-layered: compact endochorion and complex exochorion composed of air chambers. The egg capsule comprises three specialized regions of different structure: micropylar apparatus, the main part of the egg covered with ridges and the posterior pole with attachment disc. The distinct regions of the egg capsule are produced by different groups of follicular cells.

03-108

WOUND HEALING PROCESS OF THE INTEGUMENT IN THE SPIDER, *PARDOSA ASTRIGERA* (ARANEAE: LYCOSIDAE)

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The integument of cephalothorax in the wolf spider, *Pardosa astrigera*, was consist of outer cuticle and a epidermal cell layer. After puncturing of the integument by sharp needle, the wounds were gradually healed along with the following two processes: (1) Coagulation and wound plug formation by hemocytic migration, (2) Regeneration of the epidermal cell layer and secretion of a new cuticle.

Shedding of the hemolymph was immediately blocked by the coagulation, and formation of the wound plug was initiated by the accumulation of hemocytes at the wounding site. This step was accomplished within 12 hours after wounding, and it was found that the granulocyte was the most predominant type among the several types of hemocytes.

Reconstruction of the epidermal cell layer beneath the wound plug was first detected at 10 day sample, and production of new cuticle through the apical surface of the epidermal cells appeared at 14 days after wounding.

03-110

THE FUNCTION, MECHANICS AND DESIGN OF HINDWING FANS.

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The hindwings of most flying Orthoptera, Phasmida and Dictyoptera appear to gain most aerodynamic force from the downstroke. This comes partly from the initial 'peel', but also from the main translational phase, as the wing sweeps downward and forward. The fan appears subtly designed to maximise lift by a semi-automatic mechanism, the 'umbrella effect', which creates camber and depresses the trailing edge by compressing and slightly buckling the radiating veins. This is made possible by the fan shape, which approximates to part of a logarithmic spiral, and can be modelled as such both physically and mathematically to predict a theoretical optimal design. The great variability which actually occurs seems less determined by fine aerodynamic and energetic considerations than by the need to maintain an adequate area of total lifting surface in insects whose forewings are adapted to variously degrees for protection, rather than flight.

The fans of locusts, which are spectacular long-distance fliers, show many refinements on the basic form. These appear to enhance the umbrella effect, while minimising the energy expended in creating it.

03-111

Ultrastructure of digitiform sensilla on the hypopharynx of larval *Elodes marginata* FABR. 1798 and *Flavohelodes flavicollis* KIESW. 1859 (Coleoptera: Scirtidae).

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Sensilla are called digitiform if their hairshaft lies in a longitudinal and superficial groove built by the cuticle. Digitiform sensilla are widespread in different orders of insects like Coleoptera, Lepidoptera and Diptera. These sensilla are found on the antenna and the maxillar- and labialpalpi in larvae and imagines. However in larval *Elodes* and *Flavohelodes* digitiform sensilla are only found on the hypopharynx. Therefore it is important to know if such sensilla have always the same ultrastructure and function.

The four digitiform sensilla on the keel-sclerite of the hypopharynx of larval *Elodes marginata* and *Flavohelodes flavicollis* were investigated by electron microscopy.

In the two species the digitiform sensilla don't show any structural divergences of their ultrastructure. Each sensillum consists of a poreless, massive hairshaft, which lies in the superficial, cuticular groove. The basis of this peg is surrounded by typical joint-structures. At its tip the outer dendritic segment contains the tubular body and terminates on the basis of the hairshaft. A single bipolar sensory cell innervates each sensillum.

The tubular body demonstrates that digitiform sensilla on the hypopharynx in larval *Flavohelodes flavicollis* and *Elodes marginata* are mechanosensitive sensilla.

Following conclusion can be made:

In literature the term "digitiform sensilla" bases only on the external cuticular morphology of these sensilla. However our results proof that this term unites a multitude of sensilla of different types and functions.

03-113

THE ANTENNAL CIRCULATORY ORGANS OF HYMENOPTERA: FUNCTIONAL MORPHOLOGY AND PHYLOGENETIC IMPLICATIONS

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The antennal circulatory organs of 38 species of Hymenoptera were investigated by light and electron microscopy. In *Apis* this organ consists of an unpaired ampulla from which blood vessels originate extending into the antennae. The ampulla is a very delicate structure of elastic connective tissue. It connects to the head hemocoel via numerous small wall perforations. No specific ampulla muscles exist, but contraction of the pharynx dilators causes ampulla compression, thereby forcing hemolymph flow into the antennae.

The functional type of antennal circulatory organ without directly-acting ampulla muscles is common to all other Hymenoptera. In some anatomical characters, however, these organs vary considerably between taxa. There are also species with two ampullae; some have a hemolymph channel connecting the ampulla with the dorsal vessel; others have a pumping case for the ampulla formed by cuticular apophyses. There are also differences in number and morphology of the ampulla openings. The distribution of all these characters and the implications for phylogenetic systematics of Hymenoptera are discussed.

03-112

THE MORPHOLOGY OF THE CIRCULATORY ORGANS IN DIPLURA: THE ANCESTRAL CHARACTER STATE IN INSECTS?

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In contrast to pterygote insects, the circulatory organs of Diplura show a number of characters which demand special interest.

Diplura are unique in comparison to all other insects in their antennal vessels being connected to the anterior end of the dorsal vessel. Most insects possess pulsatile organs at the base of the antennal vessels which work independently of the dorsal vessel. In all subtaxa of the myriapods, there is a connection of the antennal vessels to the dorsal heart comparable to that of Diplura. This is considered the plesiomorphic state of the Tracheata. The connection of those vessels was apparently lost during the evolution of the Ectognatha.

Another remarkable feature of the circulatory system in the head capsule is a vessel ring which encompasses the gut and connects the dorsal vessel to a short ventral vessel. This feature is shared by Diplura, Archaeognatha and Zygentoma but not by any pterygote insect. A similar construction was found in Chilopoda and the possible homology of these structure is discussed.

Modifications in the morphology of the posterior end of the dorsal vessel and in the auxiliary guiding structures which take part in the hemolymph supply of the different abdominal appendages were found in Diplura, Archaeognatha, Zygentoma and Ephemeroptera. This contribution sets out to scrutinize the evolutionary pathways and transformations of these circulatory organs.

03-114

WING CIRCULATORY ORGANS AND POSTEMBRYONIC DEVELOPMENT OF THE DORSAL VESSEL IN THE THORAX OF NEUROPTERIDA (INSECTA).

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The auxiliary pumps of insect wings consist of a cuticular part forming the pump casing and of a pulsatile pumping structure. The present investigation compares the wing circulatory organs and the course of the dorsal vessel in the thorax on the basis of semithin serial sections in 32 species of Megaloptera, Raphidioptera and Neuroptera.

All Megaloptera have a pulsatile diaphragm in each pterothoracic segment which is distant from the dorsal vessel. The latter bears no ostia. In the Raphidioptera, the wing circulatory organ in the metathorax is different from that in the mesothorax. In the metathorax, the dorsal vessel has an ampullary enlargement with a pair of ostia close to the pulsatile diaphragm. In the mesothorax, the dorsal vessel is distant from the pulsatile diaphragm and has no ostia. In most investigated Neuroptera, the pulsatile diaphragms in both winged segments are directly attached to the dorsal vessel, which has a pair of ostia. In a few species, however, the pulsatile diaphragm is separate from the dorsal vessel.

Studies of the postembryonic development reveal that larval instars and imagines are comparable with regard to the course of the dorsal vessel and the occurrence of ostia.

The phylogenetic pathways of the wing circulatory organs exhibit several convergent transformations toward the appearance of separate pulsatile diaphragms. Spatial and developmental constraints are discussed as possible reasons for the evolutionary diversification of wing circulatory organs.

03-115

THE SEGMENTAL BLOOD VESSELS OF *PERIPLANETA AMERICANA*B. Mayer¹, G. PassDepartment of Evolutionary Biology, Institute of Zoology,
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Segmental blood vessels are lateral arteries of the dorsal heart tube which occur in the Blattodea and in some Mantodea. *Periplaneta americana* has a pair of short vessels in the meso- and metathorax and longer paired vessels in the 3rd to the 6th abdominal segments. The functional morphology of the heart and the segmental vessels is analyzed by light and electron microscopy. The segmental blood vessels lie entirely in the pericardial sinus and have no direct connection to the perivisceral sinus. They originate from the ventrolateral walls of the dorsal vessel and extend laterally, finally ending near the pleurae in the fat body. At first glance the segmental vessels appear as simple hemolymph spaces in the fat body, but they have a very delicate wall of connective tissue. While the dorsal vessel wall is muscular, no muscle fibers could be detected in the segmental vessels. At their origin there is a clump of loosely-packed, irregular cells which clearly have the function of a valve. *In vivo* observations of the activity of the dorsal vessel and the hemolymph flow through the segmental vessels indicate that the valves open independently of each other and show no correlation to the contractions of the dorsal vessel. The results are interpreted with regard to both functional and phylogenetic aspects.

03-117

THE ULTRASTRUCTURE OF THE GUT AND THE PERITROPHIC MEMBRANE IN FEMALES OF THE TICK *IXODES RICINUS*

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Many blood sucking arthropods possess a peritrophic membrane. Generally, ticks do not produce a peritrophic membrane except in the argasid tick *Ornithodoros moubata* (Grandjean, 1984) and several *Ixodes* species: *Ixodes ricinus* (Samson, 1909, Zhu et al., 1991, Zhu et al., 1993), *I. scapularis* (Rudzińska et al., 1982), *I. persulcatus*, *I. hexagonus*, *I. trianguliceps*, and *I. uriae* (preliminary results: Gern et al., 1995). The peritrophic membrane is thought to constitute a mechanical barrier for the penetration of pathogens such as the spirochete *Borrelia burgdorferi* (Hayes and Burgdorfer, 1992).

Using SEM and TEM electron microscopy, we reexamined in detail the structure of the gut and the peritrophic membrane in females of *I. ricinus* during feeding and the subsequent pre- and oviposition period. Special attention was paid to the foregut-midgut junction, the hindgut, and to the deposition and subsequent fate of the peritrophic membrane. The first signs of synthesis and deposition of the membrane appear earlier than the 18 hours after beginning of feeding previously reported by Zhu et al. (1991). The formation of the membrane is closely associated with the microvilli.

We hope that our studies will lead to a better understanding of the role(s) of the peritrophic membrane for the behaviour of pathogens in this tick.

03-116

HISTOCHEMICAL STUDY OF QUEENS SPERMATHECA OF *Atta sexdens rubropilosa* ANTS (HYMENOPTERA:FORMICIDAE)

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The spermatheca is a structure found in insects whose function is to store sperm from the time of copulation to time of egg fertilization. It consists of a pouch internally lined with a cuticle and frequently presenting associated spermathecal glands responsible for the production of spermathecal fluid.

The objective of the present study was to perform a morpho-histochemical study of the spermatheca of queens of *Atta sexdens rubropilosa* ants involving tests for the detection of lipids, proteins and polysaccharides. The material was fixed in 4% paraformaldehyde and embedded in JB4 resin (Polaron Instruments Bio Rad) for histological processing.

The spermatheca of the queen has the morphology of a bilobate pouch whose lobes fuse in the middle portion of the structure. No secretory gland was detected in association with the spermatheca, but groups of cells with secretory characteristics (one group in each lobe) forming a sort of acinus were observed. The histochemical tests applied revealed that the spermathecal secretion at the intracellular level is basically of a protein and polysaccharide nature in these insects. This secretion, however, may be modified in the pathway from the secretory cell to the spermathecal lumen. The storage portion of the spermatheca has thin walls consisting of epithelial cells and lined internally with a cuticle. This epithelium reacted positively to most of the histochemical tests applied. Some investigators suggest that the reduced thickness of the reservoir wall may permit the passage of products other than those secreted by the glandular cells of the spermatheca.

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03-118

DEPENDENCE BETWEEN HEART BEAT, GAS EXCHANGE RHYTHMS AND BODY MOVEMENTS IN *GALLERIA MELLONELLA* PUPAU. Tartes, A. Kuusik¹, A. Vanatoa²

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During pupal development in *Galleria mellonella* pupae there exist clear periods of rhythmic abdominal movements described as bending movements in *Tenebrio molitor* pupa (Tartes & Kuusik, 1994, *Physiol. Entomol.*, **19**, 216-222). These abdominal movements are independent from gas exchange cycles. Besides, in *Galleria mellonella* there exist irregular abdominal rotations and often gas exchange is abolished by abdominal rotation movements.

In young and middle-age pupae the heart activity periods alternate with the heartbeat pauses. In late pupae the heartbeating is almost incessant and sometimes is interrupted by short (5-6 s) pauses while common pattern is the alternation of the periods of slow and fast heartbeats and also small and large systole amplitudes.

In most middle-age and in few young pupae more than 80% of heart activity periods typically coincide with the bouts of abdominal pulses. In older pupae often heartbeat and body movements are strictly synchronized.

It could be suggested, that phenomenon, where contractions of abdominal muscles support the heartbeat, is widespread among insects. The supposed function of rhythmic body movements to support circulation may be an example of economic exploitation of the same movement for different tasks in insect.

03-119

MORPHOLOGICAL TYPES AND PHYSIOLOGICAL RESPONSES OF ANTENNAL OLFACTORY RECEPTOR NEURONS IN THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* (ORTHOPTERA: ACRIDIDAE)

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Morphological features of antennal sensilla of the desert locust, *Schistocerca gregaria*, were investigated with scanning and transmission electron micrographic techniques. Three types of olfactory sensilla were identified: sensilla coeloconica, sensilla basiconica and sensilla trichodea. S. coeloconica were situated in cuticular pits in the antennal surface. They contained one-to-four sensory neurons and double walls penetrated by slit-like pores. S. coeloconica showed high excitatory responses in a dose-dependent manner to some plant odours and organic acids that have recently been identified from odours emitted by locust nymphs. Conversely, this sensillum type was inhibited by most semiochemicals produced by the locusts and shown to affect the gregarization behaviour. S. basiconica contained up to 50 sensory neurons and each neuron displayed massive dendritic branching towards the distal end. The sensillum wall was penetrated by a high number of pores. In contrast, s. trichodea contained one-to-three sensory neurons that sent unbranched dendrites into the sensillum lumen and the sensillum wall was penetrated by relatively fewer pores. Both s. basiconica and s. trichodea showed excitatory responses to gregarization pheromone compounds and plant odours but were not affected by the organic acids.

03-121

CARDIAC ACTIVITY OF THE ADULT BUDWORM, *HELIOTHIS VIRESCENS*

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The present investigation aims at determining the most significant parameters of cardiac activity in the adult budworm, *Heliothis virescens*. Monopolar extracellular recordings of electrophysiological heart activity were obtained by using metal electrodes positioned on the dorsal cuticular body surface of intact, restrained specimens. Signals were led off via a negative-capacity amplifier, displayed on a CRO and stored on a magnetic recorder. As in the case of other insect species (Angioy and Pietra, 1995; Ai and Kuwasawa, 1995), the regular cardiac activity in *Heliothis* consists of the cyclic alternance of a fast phase with a slow phase, which are distinguishable on the basis of their action potential frequencies and durations. The fast phase comprises a high-frequency action potential discharge (135.60 ± 0.03 pulses/min) lasting 36.72 ± 8.65 s. The slow phase consists of a low-frequency action potential discharge (59.40 ± 0.06 pulses/min) lasting 64.44 ± 15.19 s. The interspike interval gradually increases from the beginning to the end of the fast phase, while it decreases in the first two-thirds of the slow phase and thereafter increases in the final third of the phase. The regular action potential frequency of each phase is greatly affected following stimulation of external sensory receptors located on several areas of the insect body. Time-course analysis of consequent electrocardiographic changes indicates that, as in the case of other insect species (Angioy et al., 1987; Ai and Kuwasawa, 1995), sensory-induced cardiac responses occur in the adult budworm, *Heliothis virescens*. Angioy A.M. (1988) J. Insect Physiol. **34**, 21-27; Angioy A.M. and Pietra P. (1995) J. Comp. Physiol. B **165**, 165-170; Ai H. and Kuwasawa K. (1995) J. Insect Physiol. **41**, 1119-1131.

03-120

ACCESSORY PULSATILE ORGANS: MODEL SYSTEMS FOR NEUROBIOLOGICAL AND EVOLUTIONARY STUDIES

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Compared to the closed vascular system of vertebrates, the open circulatory system of insects seems simple. The hemolymph is propelled through the body cavity by the tubular dorsal heart. The action of this pump alone, however, cannot effect circulation in long body appendages such as antennae, legs, wings, ovipositors, and cerci. Many insects possess small pulsatile organs for hemolymph exchange in these structures, which in most cases are completely independent from the dorsal vessel.

Comparative studies of the functional morphology of these accessory pulsatile organs in representatives of most insect orders revealed an unexpected diversity of construction. For this reason, these organs are significant for phylogenetic systematics and for general aspects of organ evolution. The reconstruction of their evolutionary pathways elucidates their origin by the recruitment of the individual components from different organ systems and the formation of new functional units. In several cases, their structural transformation and individualization can be explained by modifications of other organ systems.

Due to their capability of autonomous rhythm generation and the fact that they consist of only very few components, the accessory pulsatile organs are also ideal objects for studying the function of simple nerve-muscle systems.

03-122

MORPHOLOGY OF THE TUBULAR HEART OF THE BLOWFLY *PROTOPHORMIA TERRAENOVAE*

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Several research studies have been performed on cardiac activity and its sensory-induced changes in blowflies (Thon, 1980; Angioy and Pietra, 1995). However, very few morphological data of the contractile organ have been described in these insect species (Jensen, 1977). We report herein the results of a laser scanning confocal microscopy study on the vessel of the adult fly, *Protophormia terraenovae*. The abdominal portion of the pulsating dorsal vessel, arbitrarily called the heart, is made up of chambers in series. They are provided with lateral valve-like openings, resembling the incurrent ostia already described in other insect species. The heart wall is richly supplied by a widespread network of tracheoles deeply embedded among myocardial cells. The abdominal portion of the vessel continues with a cylindric thoraco-cephalic part, generally called the aorta, six to eight times smaller in diameter than the abdominal heart. Neither ostia nor tracheoles are present in the thoracic aorta, which extends to the head and terminates with an enlarged opening into the brain. The vessel wall consists of a layer of muscle fibers between two sheets of connective tissue. Myocytes show large nuclei, which protrude externally and internally with respect to the vessel wall in the aorta and in the abdominal heart respectively. The outer and inner perimysial layers are quite similar to those described in Orthoptera (Baccetti and Bigliardi, 1969) and contain longitudinal elastic-like and circular periodic banded collagenous-like fibrils respectively. Angioy A.M. and Pietra P. (1995) J.Comp. Physiol. B **165**, 165-170; Baccetti B. and Bigliardi E. (1969) Z. Zellforsch. **99**, 13-24; Jensen H. (1977) Cell Tiss. Res. **180**, 293-302; Thon B. (1982) J. Insect Physiol. **28**, 411-416.

Section 4

Reproduction and Development

04-001

THE EVOLUTIONARY AND ECOLOGICAL SIGNIFICANCE OF METAMORPHOSIS AND ITS HORMONAL REGULATION.

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Insect evolution is characterized by progressive divergence in the morphology of larval and adult stages. Metamorphosis is the process by which a larval form with one set of adaptations transforms into an adult form with a different set of adaptations. In addition to the sequential changes of metamorphosis, many insects can develop into one of several distinct alternative morphologies at some stage in their life cycle, each with unique specializations. The distinctive forms that develop in these sequential and alternative polyphenisms are genetically identical. They arise through discrete switches in developmental pathways, usually in response to specific stimuli from the environment. The endocrine system provides the transduction mechanism between the environmental signals and the developmental pathways. Juvenile hormones, ecdysteroids, and neurohormones are known to be involved in the regulation of these developmental switches.

Switching of developmental pathways occurs during relatively brief hormone-sensitive periods. Typically one of two alternative pathways is chosen, depending on whether the hormone is above or below a given threshold. When a choice must be made between more than two possible developmental alternatives this is generally managed through a temporal sequence of several binary switching events. This flexible and modular developmental regulatory mechanism has enabled the great evolutionary radiation of insects, and will be discussed in the context of metamorphosis, caste determination in social insects, and horn polymorphism in scarab beetles.

04-002

THE ENDOCRINE REGULATION OF THE PROCESS OF ECDYSIS DURING METAMORPHOSIS

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In *Manduca sexta* the injection of eclosion hormone (EH) into molting animals triggers the sequential performance of the pre-ecdysis and ecdysis behaviors. Part of this response to EH treatment includes the production of cyclic GMP in a group of 50 neurons that contain crustacean cardioactive peptide (CCAP). For larval ecdysis this increase occurs well into pre-ecdysis behavior, about 10-15 min before ecdysis, and increases the excitability of these cells. The number of cells showing the cGMP increase as well as its timing relative to ecdysis varies according to metamorphic stage.

The role of EH in triggering ecdysis-related events needed to be reexamined in light of the recent discovery of the ecdysis triggering hormone (ETH; Zitnan et al., *Science*, 1996). A careful determination of the timing of EH release revealed that the major release of this peptide occurs well into the pre-ecdysis behavior, at the time when the CCAP cells show their increase in cGMP. This timing also coincides with that of ETH release. Our current data suggest that EH and ETH have a positive feed-back relationship which insures the massive release of both hormones. This "spike" of peptides would provide an unequivocal signal that commits the animal to attempting ecdysis.

04-003

THE ROLE OF NEUROPEPTIDES IN METAMORPHOSIS

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It is well known that insect molting and metamorphosis are regulated by various neuropeptides. During past 10 years, these neuropeptide, such as prothoracicotropic hormone, eclosion hormone, allatotropin and allatostatin have been purified and chemically characterized. Furthermore, the cDNAs and/or genomic DNAs of these hormones have cloned and sequenced. In this talk, Our research on these neuropeptides is reviewed.

04-004

CASTES AND METAMORPHOSIS

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In social insects, individuals belonging to a same species and a same sex, display remarkable differences in their morphology (polymorphism) and/or in their behavior (polyethism). The determination of these differences occurs at critical periods during the development under the influence of morphogenetic hormones (i.e. ecdysteroids and juvenile hormone). The presentation will survey the role of juvenile hormone and ecdysteroids on caste specific features of various social insects. We shall focus on reviewing our present knowledge on the timing of hormone action in epigenetic determination of morphology and behavior.

04-006

TRANSCRIPTIONAL ANALYSIS OF JUVENILE HORMONE SENSITIVE GENES DURING INSECT METAMORPHOSIS

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The promoters of juvenile hormone (JH)-sensitive, and one JH-insensitive gene were isolated from *Trichoplusia ni*, and sequences necessary for, or affecting, transcriptional activity were identified by biochemical and functional methods. The transcription start point for each of the genes were determined biochemically, by both primer extension and the sequencing of multiple, independent full-length cDNA clones. The function of each inferred transcription start point as an actual transcription start point was confirmed by in vitro transcription assay. Using the in vitro transcription system, a minimal core promoter of 60 bp (bp -34 to +24) of the *BJHSP1* (basic JH suppressible protein 1) gene, containing a single TATA box motif approximately 30 bp upstream of the transcription start point, was functionally sufficient to support alpha-amanitin-sensitive transcription. The same construct was also transcriptionally functional in a homologous cell line transfection assay. The corresponding region of the other hexamerin genes also contains a similarly positioned TATA box motif, and promoter constructs for each that included the transcription start point, initiator and inferred basal transcription apparatus binding site were all transcriptionally functional in cell line transfection assay. The action of sequences 5' to the minimal promoter region in modulating the rate of transcription was shown by a cell line transfection assay of a nested deletion series of the promoter for the gene encoding BJHSP1, in which the results identified a strongly suppressive element between positions -160 and -109. This system of genes, including those sensitive to JH and one not sensitive, should be useful in a comparative approach toward identifying those regulatory motifs that are functionally necessary to transduce the regulatory action of JH.

04-005

REGULATION OF THE ECDYSONE-INDUCED CASCADE IN *MANDUCA* AT METAMORPHOSIS BY JUVENILE HORMONE

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The polymorphic epidermis of the tobacco hornworm makes first several larval cuticles, then at metamorphosis switches and produces a pupal cuticle. When day 2 5th instar larval epidermis was exposed to 500 ng/ml 20-hydroxyecdysone (20E) for 24 h *in vitro*, all cells became pupally committed. The presence of juvenile hormone (JH) prevented this switch. During the exposure to 20E alone, the mRNAs for both isoforms of the ecdysone receptor (EcR) and for E75A showed a biphasic increase, first peaking at 3-6 h, then rising again between 12 and 24 h. The constitutive Ultraspiracle (USP) mRNA showed no change until after 6 h, then increased. Broad-Complex (BR-C) mRNA first appeared at 6 h, then increased steadily. E75B mRNA showed no change. A comparison of the cellular changes during this period with those occurring *in vivo* during pupal commitment in response to 75 ng/ml 20E equivalents showed that the initial responses are those seen during the commitment phase whereas by 18-24 h the cells are beginning to show the predifferentiative changes associated with the prepupal rise of ecdysteroid. In the presence of 1 µg/ml JH I or methoprene, the initial increases in EcR-A and -B1 mRNAs were unchanged, but the second rise was prevented as was the later rise in USP mRNA. Also, the appearance of BR-C mRNA was prevented in these larvally committed cells. By contrast, the initial increase in E75A mRNA was nearly twofold higher, and this increased level was maintained throughout the culture. Initial experiments indicate that the higher level of E75A mRNA is due to an increased transcription rate in the presence of JH and that inhibition of protein synthesis during pretreatment with JH can block this effect. Thus, the role of JH is to modify some early responses to 20E which prevent the later appearance of metamorphic-specific transcription factors such as BR-C.

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04-007

Mode of action of RH-5992(Ecdysteroid agonist) in pest control operations

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Upon ingestion by lepidopteran larvae, RH-5992 induces an incomplete precocious molt which results in the death of the insect. Similar to the natural hormone, this agonist binds to the ecdysteroid receptor and initiates a cascade of gene expression, if the larva is exposed to this compound early in the stadium before ecdysteroid secretion. If the larva feeds on this material later in the stadium after the ecdysteroid peak, there is no immediate molt induction but the effect is manifested early in the subsequent larval stadium. The spruce budworm, *Choristoneura fumiferana* has six larval instars and all of these stages are sensitive to RH-5992. No phenotypic effects can be seen in the pupal and adult stages. Of all the larval instars, the second instar which is the stage in which this insect overwinters, is the most sensitive stage to RH-5992. The transcription factor, CHR3, is expressed in response to either RH-5992 treatment or at the onset of ecdysteroid secretion and has been routinely used as an assay to detect the initiation of molting. The diapausing second instar does not express CHR3 throughout the stadium but when exposed to even extremely low levels of RH-5992 this gene is expressed. Ecdysteroid receptor is present in low levels throughout development but no ecdysteroid is secreted during the diapausing second instar larva, making this stage very responsive to this agonist.

Since within 12 hr after ingestion of RH-5992 the larva goes into the molting phase, one of the earliest physiological effects is the cessation of feeding. This is very significant in terms of its control potential because it limits the destruction of the host. The lepidopteran specificity combined with feeding inhibition makes this compound an environmentally attractive control agent. Some examples of its proposed use will be illustrated with results from field trials.

04-008

PEST CONTROL BY INTERFERING WITH MOLTING AND METAMORPHOSIS

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The steroid hormone, ecdysone, initiates and coordinates molting and metamorphosis through a cascade of gene expression and repression events. Like other steroid hormones, ecdysone acts through intracellular receptors at the transcriptional level. The stable ecdysteroid analog RH-5992 controls insects by interfering with the process of molting. To understand the mode of action of this compound and to verify whether it is possible to interfere with molting and metamorphosis by either over or under expressing one or more of the key regulatory molecules, we recently cloned three members of the steroid hormone receptor superfamily: *Choristoneura* ecdysone receptor (CfEcR), *Choristoneura* hormone receptor 2 (CHR2) and *Choristoneura* hormone receptor 3 (CHR3) from the spruce budworm, *Choristoneura fumiferana*. The mRNAs for these three receptors increase in abundance during the ecdysteroid peaks for embryonic, larval, pupal and adult molts but not during the intermolt periods. Upon feeding RH-5992 induces the expression of CfEcR, CHR2 and CHR3 mRNA in midgut, fat body and epidermis of *C. fumiferana*. The induction pattern for each of these receptors is similar in both *in vitro* using 20E and *in vivo* using RH-5992. However, the time course of induction for each of these receptors is different. CfEcR was induced within an hour of exposure to 20E or RH-5992, and reached maximum levels by 3 hr following which it decreased to normal levels by 6 hr. CHR2 mRNA was induced within an hour after exposure to 20E or RH-5992, and reached maximum levels by 3 hr followed by a decrease after continuous exposure for 6 hr. CHR3 mRNA started increasing at 3 hr and rose to a maximum level in 6 hr. After continuous exposure to 20E or RH-5992 for 12 hr, the mRNA levels started declining. Supported by the Canadian Forest Service and the National Biotechnology Strategy fund.

04-009

SEX DETERMINATION IN DIPTERANS AND THE ROLE OF SEX-LETHAL

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Among dipterans, different strategies are employed to select and stably maintain male or female development. Nevertheless, these strategies may be based on a common principle in which a primary signal controls the activity of a binary switch gene (1). In the best studied system, *Drosophila melanogaster*, the *Sxl* gene assumes this switch function. When ON, it imposes female development; when OFF, male development ensues. Likewise, genetic studies in the housefly, *Musca domestica*, have identified a putative master switch gene *F* which directs female development when ON, and male development when OFF (2).

We have investigated if the *F* and *Sxl* switch functions are founded on common molecular grounds. Analysis of a *Sxl* homologue in the housefly reveals a high degree of structural similarity and, when introduced into *Drosophila*, shows conservation of the molecular functions. However, the *Sxl* gene is not subject to sex-specific regulation in *Musca*. Thus, this gene cannot act as a switch in sex determination of the housefly and does not correspond to *F*. In *D. virilis*, and in contrast to *D. melanogaster*, the *Sxl* homologue expresses large amounts of a male-specific protein variant in the developing CNS of male embryos (3). It is thus conceivable that this gene may also perform functions not related to sex determination. *Sxl* may be an example for the functionally divergent evolution of a regulatory molecule.

(1) Nöthiger and Steinmann-Zwicky (1985) Cold Spring Harbor Symp. Quant. Biol. 50, 615-621

(2) Dübendorfer et al. (1992), Sem. in Devel. Biol. 3, 349-356

(3) Bopp et al. (1996), Development 122-03

04-010

SEARCHING FOR SEX DETERMINING GENES IN *CERATITIS CAPITATA*: ANALYSIS OF GENES HOMOLOGOUS TO *DROSOPHILA MELANOGASTER* SEX-LETHAL (*SXL*) AND *DOUBLESEX* (*DSX*).

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In *D. melanogaster*, sexual development is controlled by a hierarchical cascade of regulatory genes (*Sxl* > *transformer* > *dsx*) and regulation of these genes involves sex-specific RNA processing. As in no other Diptera (neither Arthropoda) the molecular basis of sex determination has been elucidated yet, we focused our studies on the Mediterranean fruit fly, *C. capitata* (Diptera), also known as an important agricultural pest. We isolated the homologue of *Sxl* in *C. capitata* (*CcSxl*) and found that this gene encodes for highly conserved protein-products (79% of similarity). In contrast to *Drosophila*, however, this gene is not sex-specifically regulated, but produces the same gene-products in males and females. Therefore, we focused our attention on the bifunctional gene *dsx* which in *Drosophila* acts at the bottom of the *Drosophila* sex regulatory cascade. *dsx* in *Drosophila* encodes sex-specific proteins with opposing functions in sexual differentiation. Male and female-specific proteins share a common amino-terminal part which contains a zinc-finger DNA binding domain, but differ in sequence at the carboxy-terminal ends. We isolated sequences from *C. capitata* which are homologous to *dsx* in *D. melanogaster*. Sequence analysis of an ovarian, a female adults cDNAs and a genomic clone show a high percentage of identity at the nucleotide and amino acid level. The cDNA clones contain coding sequences at the 3' end which correspond in composition to those in female-specific *dsx* transcripts in *D. melanogaster*. Also, *Ccdsx* encodes a conserved zinc finger domain in the amino-terminal part of its gene-products. Preliminary RNA analysis of males and females suggests the presence of sex-specific transcripts in *C. capitata* indicating that *Ccdsx* may perform sex-specific functions. Germline transformation experiments are in progress to test whether *Ccdsx* acts as a bifunctional switch gene in sexual development of *Ceratitis*. These data will be discussed in the light of the "bottom-up" hypothesis of how sex determination pathways have evolved (Wilkins, *BioEssays* 17 no. 1, 71-77).

04-011

SEX-PEPTIDE REGULATES VITELLOGENIC OOCYTE PROGRESSION VIA JUVENILE HORMONE.

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Mating elicits two well defined responses in females of *Drosophila* and other insects: receptivity is reduced and oviposition is increased. Central to the control of receptivity and oviposition in *D. melanogaster* is the sex-peptide (SP), a 36 amino acid peptide synthesized in the accessory glands. During copulation it is transferred into the female. Injected, or ectopically expressed, SP induces reduced receptivity and increased oviposition, the two post-mating reactions observed after mating.

The induced changes imply that the nervous system and (neuro-)endocrine factors are involved in the response of the female to the SP. We have focused on the elucidation of regulatory mechanisms of the SP reaction cascade which lead to the maintenance of the oviposition response. Mated or SP-injected females produce large numbers of eggs which is reflected by an activation of oogenesis. The production of yolk proteins by the fat body and the ovary plays a major part in this process. Quantitative comparison of hemolymph and ovarian yolk protein levels, their rates of synthesis and the levels of yolk protein transcripts in the fat body and ovary shows that the fat body is continuously producing vitellogenin while synthesis and uptake are enhanced in the ovary. This suggests that the regulation is at the level of vitellogenic oocyte progression. Application of the juvenile hormone (JH) analogue methoprene leads to the same changes in vitellogenesis as mating or SP-injection does, indicating that JH acts downstream of SP.

04-012

EXTRAORDINARY REPRODUCTIVE AND DEVELOPMENTAL MODES IN HYBRID STICK-INSECTS WITH SUBSTITUTION, ELIMINATION, ADDITION OF WHOLE GENOMES

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The genus *Bacillus* comprises two well differentiated bisexual species which have repeatedly hybridized to produce two hybridogenetic taxa (*B. rossius-grandii benazzii*, *B. rossius-grandii grandii*) and a corresponding allodiploid parthenogen (*B. whitei* = *B. rossius/grandii grandii*); *B. rossius* has always been the maternal ancestor. Male hybridogens are sterile, whereas females are fertile. Electrophoretic gene-enzyme markers show that hybridogenetic eggs pass the whole maternal haploset (*rossius*) to progeny, whereas the paternal genome (*grandii*) is eliminated; later it is replaced through real fertilization by that of a host fathering male, so that hemiclinal hybrids are restored each generation. Genetic and chromosomal markers also demonstrate that the egg genome can be completely eliminated and an all-paternal, non-hybrid progeny of both sexes are produced through the fusion of two spermheads of the several entering the egg (androgenesis). Depending on the fathering taxon, a single female can originate androgens of different species/races. Furthermore, several hybridogenetic-androgenetic mosaic specimens are obtained; when their differently originated cell-lines are discordant for sex chromosomes, mosaics are also intersexual. Androgenetics can similarly originate from the clonal parthenogen *B. whitei*, following fertilization by *B. grandii* or *B. rossius* males. Fertilized eggs of *B. whitei* are even capable of incorporating a third genome to produce fertile "synthetic" triploids. The genome addition promotes genetic diversification in their progeny owing to a partially restored recombination in the allotriploid eggs. All this gives evidence that no genomic imprinting is at work in stick insects and it also suggests the existence of much more flexible breeding systems than usually assumed for hybrid/unisexual organisms.

04-014

WHY DO NOT DIPLOID MALES OCCUR IN THE FIELD POPULATION OF THE HAPLO-DIPLOID TURNIP SAWFLY *ATHALIA ROSAE* (HYMENOPTERA, TENTHREDINIDAE) ?
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A variety of hymenopteran species in taxonomic-ally diverse groups have a single-locus multiple-allele sex determination system and produce diploid males (homozygous for sex alleles) through sister-brother mating in the laboratory. Diploid males usually have been observed at low frequencies and never been functional. In *Athalia rosae* with a single sex locus system, we can get diploid adult males very easily from sister-brother mating without remarkable developmental mortality. However, we can not find any diploid males in the field population. My investigation has revealed that three major factors would act to obstruct the occurrence of diploid males of *A. rosae* in the field:
(1) Females emerge earlier and move out more quickly from the natal patch than their brothers. This would reduce the sib-mating among progeny.
(2) Females, in case of double matings, always use the second-male sperm for daughter production. Therefore, sib-mating, even if occurred in the natal patch, would be cancelled by the second mating with a male of other brood after her short flight.
(3) A large number of sex-alleles (about 50 in the local population near Kobe) would prevent to produce homozygotes for sex alleles in outbreeding in the field.

04-013

"COMPLIMENTARY SEX DETERMINATION, MULTIPLE MATING AND SEX ALLOCATION IN THE PARASITOID *DIADEGMA CHYROSTITICOS*."
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The solitary endoparasitoid *Diadegma chyrostiticus* shows a heavily male biased secondary sex ratio in culture, but not in the field. The establishment of isofemale lines (son x virgin mother mating and subsequent within-sibling brother x sister mating) of *Diadegma chyrostiticus* results in the rapid male bias of subsequent offspring and in the presence of diploid sons when cultured at 24-27°C, in accordance with single locus complementary sex determination (sl-CSD). However, when isofemale lines are maintained at 16-18°C neither the male biased sex ratio nor diploid sons are observed. The presence of sl-CSD is further confirmed by genetic studies using both phenotypic mutants and alloenzyme studies. At both rearing temperatures a marked inbreeding suppression of maternal fecundity is observed. As predicted, outbreeding restores both a normal maternal fecundity and sibling sex ratio in the next generation. Moreover, *Diadegma chyrostiticus* are observed *in vitro* to undergo multiple mating and to be partially pro-ovigenic. The consequences of multiple mating results in a host-dependent increased maternal fecundity but in a further male biased progeny sex ratio and reduced maternal longevity. For this parasitoid the sex ratio is further compounded by an adaptive sex ratio allocation in response to host size, superparasitism and foundress effects-.

04-015

OOGENESIS IN LOWER DIPTERANS: POTENTIAL GERM-LINE / SOMA INTERACTIONS AND EMBRYONIC POLARITY
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Oogenesis in the lower dipterans (Nematocera) differs remarkably in several respects from its brachyceran counterpart (well studied in *Drosophila*). This may be related to peculiarities in embryonic patterning and polarity (see Seminars in Developmental Biology, Vol. 7). Nematoceran oogenesis was therefore studied in the scanty literature and in some psychodid and scatopsid species, in order to help reconstructing evolutionary divergence between the two suborders. In nematocerans, the oocyte-nurse cell complex apparently polarizes without obvious reference to any somatic structure. Only thereafter are the polarized complexes settling in minute aberrant "ovarioles". The somatic follicular epithelium differs in that neither its "centripetal" cells nor the anterior polar cells (border cells in *Drosophila*) reach the anterior face of the oocyte before nurse cell regression is almost complete. Moreover, the border cells produce a massive micropylar structure while still located anterior to the nurse cells. They then carry the micropyle along to the oocyte surface very late in oogenesis; thus oocyte / polar cell interactions can only be brief but might be extended via the micropylar structure (*tsl* storage?). In *Psychoda*, unusual interactions may occur between the spermatozoa and the somatic cells of the ovariole stalk that harbour them in large numbers - perhaps to compensate in some way for sperm immotility?

04-016

CHARACTERIZATION OF NEW GENES INVOLVED
IN THE GENERATION OF DORSAL-VENTRAL
AXES OF DROSOPHILA EGG AND EMBRYO

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Establishment of the dorsal-ventral axes of the *Drosophila* egg and embryo is mediated by two different, but related signal transduction pathways. One is required for the specification of dorsal follicle cell fate during oogenesis and starts with a signal from oocyte and received by the follicle cells on dorsal side. The other, for specification of dorsal-ventral cell fate during embryogenesis, starts with an unknown signal possibly from follicle cells and received by nuclei of the syncytial blastoderm. Using enhancer trap *gal4/UAS* lines, we have identified four distinctive genes which encode 1) HLH negative transcriptional factor, 2) secreted peptide, 3) tyrosine kinase, and 4) new protein. Whole mount ovary in situ hybridization analysis shows that these four genes have two quite different expression pattern. Genes 1 and 3 are expressed specifically in antiodorsal (also posterior for gene 1) follicle cells surrounding the oocyte in the stage 8-10 of oogenesis, while genes 2 and 4 are expressed in the overlaying follicle cells except antiodorsal in the stage 10-11, suggesting their involvement in two different pathways. Examination of their expression in two different mutant, *grk* and *fs(1)K10* background shows corresponding changes, the expression of gene 3 in *fs(1)K10* mutant ovaries expanded from the antiodorsal towards antioventral side, indicating that all of them function downstream of *grk* and *fs(1)K10* in the genetic hierarchy of dorsoventral axis.

04-018

REGULATION OF THE DROSOPHILA MELANOGASTER
VITELLOGENIN RECEPTOR GENE, YOLKLESS, DURING
OOGENESIS.

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In *Drosophila melanogaster*, the uptake of yolk proteins into the oocyte only occurs during certain stages of development (stages 8-10). Yolk protein uptake is first seen following the juvenile hormone induced transition from stage 7 to stage 8, while yolk uptake terminates with the completion of the vitelline membrane around the oocyte. In order to determine if regulation of the yolk protein receptor is involved in the control of yolk uptake, we examined the expression of the receptor. The receptor, encoded by the *yolkless* (*yl*) gene, is a member of the low density lipoprotein receptor superfamily.

Whole mount in situ analysis of *yl* RNA and protein revealed that the *yl* gene is expressed very early during the development of the oocyte. *yl* RNA is expressed only in the germline cells (nurse cells and oocyte) and the RNA accumulates in the presumptive oocyte as early as the germinal stages. This localization requires the activity of *BicaudalD* and *egalitarian*, which are involved in differentiating the oocyte from the nurse cells. The oocyte staining of *yl* RNA is observed through stage 8. During later stages, *yl* RNA is detected in both the nurse cells and the oocyte. The Yl protein is also apparent in the germinal stages, early during the development of the oocyte. Yet, in contrast to the *yl* RNA pattern, Yl protein is seen only in the oocyte and not in the nurse cells of later (stage 10) chambers. Thus, *yl* RNA may be translated more efficiently in the oocyte than in the nurse cells. Furthermore, until stage 7, Yl protein is distributed uniformly throughout the oocyte, but following the transition to the vitellogenic stages, Yl becomes progressively more cortical. By stage 10, the protein is almost entirely at the cortex. Immunogold electron microscopy has shown that Yl is associated with the endocytic apparatus (e.g. coated pits, vesicles, tubules). Thus, yolk uptake at stage 8 appears to be regulated through the activation and relocalization of the receptor or other endocytic components.

We are currently examining the promoter elements required for transcription in the germline and we are examining the relocalization of the receptor following the onset of vitellogenesis.

04-017

MOLECULAR ANALYSIS OF RECEPTOR-ENDOCYTIC
MACHINERY IN MOSQUITO OOCYTES

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Insect oocytes are highly specialized for receptor-mediated endocytosis of yolk protein precursors, and are characterized by a concentration of endocytic organelles unparalleled in any other cell. To study the biogenesis of the endocytic machinery in mosquito oocytes at the molecular level, we cloned and analyzed cDNAs and genes encoding two major coated vesicle components: 180-kDa clathrin heavy chain (CHC) and 205-kDa vitellogenin receptor (VgR). In the mosquito CHC gene, which spans more than 25 kb, the coding region is divided into seven exons, five of which encode the protein. Alternative first exons are used differentially for the oocyte-specific and somatic transcripts. Although the 3'-untranslated regions of both these transcripts are encoded by the same last exon, located 12 kb apart from others, the 3' region of the somatic 7.5-kb transcript is 1 kb longer than that of the oocyte-specific 6.5-kb transcript. The structure of the 3'-untranslated regions suggests that the oocyte-specific transcript is more stable than the somatic transcript. The 7.3-kb VgR cDNA encodes a 203-kDa protein which belongs to the low-density-lipoprotein receptor family. In situ hybridization, Northern, and slot-blot analyses shows that these genes are expressed similarly: both CHC and VgR transcripts are evident in the germ cells very early during differentiation of primary and secondary follicles. In pre- and vitellogenic follicles, the transcripts were present in very high concentration in developing oocytes and only in trace amount in nurse cells.

04-019

SOMATIC CELL AND GERM CELL REGULATION OF OOGENESIS IN
PHORMIA REGINA (MEIGEN)

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In the black blow fly, *Phormia regina*, oogenesis is regulated by factors (or actions) from both somatic and germ cells like in the case of other insects. These cells include median neurosecretory cells of the pars intercerebralis, corpus allatum cells, fat body cells, follicular cells, and the oocyte itself. More recently, we have discovered that endocrine cells in the midgut will secrete substances into the hemolymph in liver-fed flies 6 h after the protein meal.

The sequence of events and the action of the involved cells are described below. A protein meal will cause the release of substances from the midgut. Results suggested that two substances are released simultaneously. Both substances can stimulate oogenesis in the bioassay our used. Two hours after the release of these midgut substances, the volume of the median neurosecretory cells (A-type) increased to the maximum before decreased to the pre-protein meal level 4 h later. It appeared that this neurosecretory activity, as reflected by the volume change of A-cells, in turn turned on the ecdysteroid production/release by the ovaries and the juvenoid production/release by the corpus allatum.

In response to ovarian ecdysteroid, competent fat body cells produce/secrete vitellogenin into the hemolymph. In response to juvenoids, follicle cells change shape to make intercellular space (patency). Meanwhile, oocytes begin to produce vitellogenin receptor which is required to form the vitellogenin-specific coated pits to allow endocytosis of vitellogenin into the developing oocyte. What governs the production of vitellogenin receptor is unclear in *P. regina*. It is obvious that we have long way to go before fully understand all the mechanisms governing oogenesis in this blow fly.

04-020

ORIGINS OF ANTERIOR-POSTERIOR AND DORSAL-VENTRAL POLARITY DURING OOGENESIS IN DROSOPHILA

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In *Drosophila*, the dorsal-ventral polarity of the egg chamber depends on the localization of the oocyte nucleus and the *gurken* RNA to the dorsal-anterior corner of the oocyte. The TGF α -like *gurken* protein presumably acts as a ligand for the *Drosophila* EGF receptor (*torpedo/DER*) expressed in the somatic follicle cells which surround the oocyte. We have identified *cornichon*, a new gene required in the germline for *gurken-torpedo* signaling. A phenotypic analysis reveals that, in addition to their role in dorsal-ventral patterning, *cornichon*, as well as *gurken* and *torpedo*, are required in an early signaling event between oocyte and posterior follicle cells which establishes the anterior-posterior polarity of the egg chamber. Mutations in all three genes prevent the formation of a correctly polarized microtubule cytoskeleton required for proper localization of the anterior and posterior determinants, *bicoid* and *oskar*, and for the asymmetric positioning of the oocyte nucleus. Therefore, *cornichon-gurken-torpedo* signaling is not only required to transmit the primary dorsal-ventral polarity established by the asymmetric positioning of the oocyte nucleus, but the signaling itself is a prerequisite to generate the primary dorsal-ventral polarity. We plan to clone *gurken* from other insect and arthropod species to study the evolution of primary axis formation and its relation to different types of oogenesis.

04-022

A POTENTIAL RECEPTOR FOR THE HEDGEHOG GENE INVOLVED IN PATTERNING OF THE DROSOPHILA EMBRYO

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ABSTRACT NOT RECEIVED

04-021

Interaction of the morphogens Bicoid and Hunchback in patterning the anterior of *Drosophila melanogaster* (Meigen)

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Removal of maternal and zygotic *hunchback* (*hb*) activity by a molecular genetic approach, localizing *nanos* RNA at the anterior pole (BBNH system), has suggested that a synergistic interaction between *hb* and *bicoid* (*bcd*) is required for anterior patterning of the *Drosophila* embryo. However, generating germline mosaic clones by the "flipase-dominant female sterile" (FLP-DFS) technique shows that *hb* activity is not necessary for anterior development.

These contradicting results can be due to different levels of *bcd* activity in the two systems, since the BBNH system interferes with it, whereas the FLP-DFS technique does not. To test the hypothesis that the interaction between *bcd* and *hb* becomes only apparent at reduced levels of *bcd* activity, we removed both *bcd* and *hb* activities with the FLP-DFS technique and varied the level of *bcd* activity by the introduction of transgenes.

Our results indicate that wildtype levels of *bcd* activity are sufficient to pattern the anterior even in the absence of *hb* activity. However, in a situation where the *bcd* activity level is reduced to a degree that it is itself insufficient to organize anterior development, *hb* activity can provide additional organizing function to rescue anterior patterning. Bicoid and Hunchback are therefore indeed able to synergize with each other in order to organize anterior development, despite that in a wildtype embryo this interaction is not required for it.

04-023

THE ROLE OF EXTRADENTICLE IN LEG DEVELOPMENT OF DROSOPHILA MELANOGASTER

S. Gonzalez Crespo (Madrid-Spain)

ABSTRACT NOT RECEIVED

04-024

PATTERN FORMATION IN POLYEMBRYONIC DEVELOPMENT: HOW IS THE INSECT BODY PLAN ESTABLISHED WITHOUT THE SYNCYTIIUM?

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Development of the polyembryonic parasitic wasp *Copidosoma floridanum*, which develops up to 2000 individuals from the single egg, dramatically deviates from the general pattern of insect development. It is unclear how an individual embryo is patterned in such an extreme form of embryogenesis. To understand the mechanisms behind polyembryonic development we first described the embryonic development of *C. floridanum* using cell labeling, phalloidin and nuclear staining. We also examined the expression of homologues of the *Drosophila* segmentation genes *even-skipped*, *engrailed* and *Ultrabithorax/Abdominal A* using monoclonal antibodies.

We have found that complete cellularisation in *C. floridanum* embryonic development takes place early in development and that each individual embryo develops from a cellularised embryonic primordium and does not have a syncytium. Eve antigen was first expressed as homogenous domain in the blastula stage embryo, from the posterior to 3/4 the length of the blastula. Following gastrulation, stripes were sequentially formed from anterior to posterior, and the expression corresponded to a segment polarity pattern and not to the pair-rule pattern described in *Drosophila*. The pair-rule expression of Eve has likely been secondarily lost in polyembryonic insects, as a consequence of establishing segmental pattern in a cellularized environment. En antigen was expressed in an anterior-to-posterior progression, without pair-rule modulation characteristic for *Drosophila*. In addition, the En expression domains overlapped with the mature Eve stripes *Ubx/Abd A* was detected in the phylotypic stage of development in the putative abdominal region, and had a conserved pattern of expression. Our results indicate that neither a syncytium nor the formation of double-segments with Even-skipped is required for segmental patterning in insects. Experiments are currently in progress to analyse pattern formation upstream of pair-rule cascade.

04-026

BIOSYNTHESIS AND ENDOCYTOSIS OF MOSQUITO VITELLOGENIN: CLONING OF PRO-VITELLOGENIN CLEAVAGE CONVERTASE AND VITELLOGENIN RECEPTOR cDNAs

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The cornerstone of insect egg maturation is massive secretion of vitellogenin (Vg), and its specific accumulation by oocytes. In the mosquito fat body, a single Vg precursor (pro-Vg) is cleaved into small and large subunits. The cleavage site of pro-Vg contains the paired-basic motif, RX(K/R)R, the recognition sequence for eukaryotic convertases. To identify the insect Vg cleavage enzyme, a cDNA encoding Vg convertase (VC) was cloned from the mosquito, *Aedes aegypti*. The 3,735 bp-long VC cDNA encodes a 115-kDa protein with high similarity to, and a domain structure typical of, furin-like convertases. Co-expression of this VC cDNA with the truncated mosquito Vg cDNA resulted in correct cleavage of pro-Vg. A 4.2-kb VC transcript is expressed in the vitellogenic fat body.

The 205-kDa vitellogenin receptor (VgR) is a key oocyte-specific molecule which mediates internalization of its ligand, Vg. Following purification and microsequencing, we cloned two cDNA fragments that encompass the entire VgR coding region. The mosquito VgR deduced amino acid sequence reveals a modular arrangement typical of the low-density-lipoprotein receptor family and shares significant homology with the chicken VgR. The mosquito VgR shows even greater homology to the *Drosophila* yolk protein receptor, in spite of their respective ligands being unrelated.

04-025

EVOLUTIONARY CONSERVATION AND DIVERGENCE OF DROSOPHILA SEGMENTATION GENES IN INSECTS

D. Tauts (Munich-Germany)

ABSTRACT NOT RECEIVED

04-027

REGULATION OF YOLK PROTEIN GENE EXPRESSION IN DROSOPHILA MELANOGASTER

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The three yolk protein genes (*yp1*, *yp2* and *yp3*) of *Drosophila melanogaster* are expressed in the adult female fat body and in the follicle cells at specific stages of oogenesis. Expression in the fat body depends upon the sex-determination pathway, the hormones 20-hydroxyecdysone (20-OHE) and juvenile hormone, the nutritional status of the fly and tissue specific factors. We will describe the results of experiments using P-element mediated transformation to investigate the cis-acting sequences required for the regulation of *yp* gene expression in the fat body. Sequences have been identified 5' of the genes which confer their tissue and sex specific expression in the fat body. Regions responsive to ecdysteroids are located 5', within the coding region and 3' of the gene.

The sex determination gene, *doublesex*, has been shown to bind to one of the cis-acting sites that directs sex-specific for the body expression of *yp1* and *yp2* (Burtis *et al* 1991, EMBO J 10: 2577). 3 other trans-acting functions bind *in vitro*, namely AEF, BBF-2 and C/EBP (Falb and Maniatis 1992, Genes Dev 6: 454) to the same DNA fragment. It is not known whether 20-OHE and its receptor act directly or indirectly on these genes. We present evidence on the mode of action of 20-OHE and show that not all of the factors which bind *in vitro* to sequences flanking the *yp* genes have a role to play *in vivo*.

04-028

FATE OF YOLK PROTEINS DURING EMBRYOGENESIS IN LEPIDOPTERA

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The role of egg proteinases in insects appear to be primarily for utilization and mobilization of yolk proteins during embryogenesis. To prevent inappropriate and uncontrolled degradation of yolk proteins and to accomplish the aims of supplying amino acids for synthesis of embryonic proteins, proteolytic activity in developing eggs are a highly regulated process, whereas despite the importance of the process, the regulatory mechanism of yolk protein degradation still remains to be solved. Very recently, we have purified cathepsin L-like proteinase (BCP) from *Bombyx* eggs and two cathepsin B-like proteinases from *Antheraea* and *Samia* eggs (ACP & SCP). They are all synthesized in extra ovarian tissues (fat bodies and follicle cells) under the control of ecdysone, and stored in the oocytes as a latent inactive proenzymes. In the case of *Bombyx* eggs, these were confirmed by means of *in vitro* translation of RNA and Northern blot analysis using cDNA as a probe. Immunohistochemical experiments also suggest this possibility. During embryogenesis, cysteine proteinases are activated by limited proteolysis. BCP is activated autocatalytic manner, and ACP and SCP required another enzymes for their activation. In order to learn more about the mechanism of activation, recombinant proBCP as well as intermediate forms of the proteinase during activation was prepared and the mechanism of activation was compared with that of purified proBCP. In this symposium, we will discuss about proteinases in the eggs of lepidopterous insects, which include biosynthesis, accumulation in the ovary, mechanism of activation, and regulatory mechanism of yolk protein degradation during embryogenesis, with special emphasis on recent results obtained from the silkworm, *Bombyx mori*.

04-029

THE PURIFICATION AND CHARACTERIZATION OF A CATHEPSIN B-LIKE PROTEINASE FROM THE EGGS OF TUSSAH, *ANTHERAEA PERNYI*Xiao-fan Zhao¹, Jin-xing Wang¹, S. Y. Takahashi², Y. Yamamoto²¹ Department of Biology, Shandong University, Jinan, P. R. China-2 Department of Biology, Faculty of Liberal Arts, Yamaguchi University, Yamaguchi, Japan

Several types of egg proteinases have been reported in insects, for example, both cysteine and serine proteinases are found in *Bombyx mori*. However, whether these proteinases exist widely and act with the same mechanism in the eggs of all Lepidoptera has not yet been determined. Recently, a cathepsin B-like proteinase was identified in the eggs of *Antheraea pernyi*. Furthermore, the enzyme was purified by chromatography. The molecular mass of the proteinase was estimated to be 47 kDa by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and 160 kDa by Sepharose CL-6B gel filtration, suggesting that the proteinase is a tetramer. The activity of the purified proteinase was strongly inhibited by N-[N-(1,3-trans-carboxyoxiran-2-carbonyl)-L-leucyl]-agmatine (E-64) and leupeptin. The pH optimum was 3.5 as determined using the bovine hemoglobin as the substrate. The results of SDS-PAGE showed that the vitellin was the main component of the yolk proteins, while the amount of 30 K protein was very low in *Antheraea pernyi*. The antisera to vitellin from *A. pernyi* could recognize vitellin from *Philosamia cynthia ricini*. Antisera to cathepsin B-like proteinase from *A. pernyi* also cross reacted with the antigen from *P. c. ricini*, which indicated out the similarities on the enzymes and substrates *in vivo* between these two species.

04-030

A COMPARATIVE ANALYSIS OF ANIMAL VITELLOGENIN GENESH. H. Hagedorn, Z. Tu and D.R. Maddison
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Sequences for 17 vitellogenin genes, from 12 different species including 6 insects, are now available. Comparison of the deduced amino acid sequences has shown that they are members of a multigene family. A conserved region has been identified that is common to the vitellogenin genes of three insects, the nematode and the vertebrates. This conserved region also aligns with the Cycloraphid Dipteran yolk proteins, lipases and an α -helix-rich sequence in the lamprey vitellogenin gene. We hypothesize that several gene duplications gave rise to the vitellogenin gene family and that the yolk proteins of the Cycloraphid Diptera were derived from the other animal vitellogenin genes.

04-031

PURIFICATION AND PARTIAL CHARACTERIZATION OF VITELLIN(S) OF THE RED COTTON STAINER, *Dysdercus koenigii***Venugopal, K.J.** and Dinesh Kumar

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The predominant yolk protein(s), vitelin(s), were purified from freshly laid eggs by high salt extraction and low salt precipitation followed by gel-filtration and ion-exchange chromatography. Vitellin(s) eluted in gradient NaCl as two distinct peaks (Vitelin A-VnA- of approximate mol. wt. 290 k Da and vitelin B-VnB- of approximate mol. wt. 260 k Da)

Native PAGE of VnA and vnB displayed single bands of respective Rf values 0.17 and 0.25, both corresponding to different proteins of the crude egg extract which revealed 4 bands of Rf values 0.12, 0.17, 0.25 and 0.28. Both the vitellins were glycolipoproteins as exhibited by their staining nature.

VnA and VnB had similar electrophoretic behaviour in SDS-PAGE in which VnA resolved into 3 polypeptides (mol. wt. Ca. 116, 92, 62 k Da) and vnB into 4 with an additional 40 k Da polypeptide; all the sub units staining positive for carbohydrates and lipids. Both the vitellins lacked interchain disulfide linkage.

Immunodiffusion tests conducted using polyclonal antibodies raised separately against the vitellins showed their mutual immunological identity. The antivitelin antisera cross reacted with mated, vitellogenic, female fat body, haemolymph and ovary but did not show any reaction with mated or 5th instar female fatbody or haemolymph exhibiting their sex and stage specificity.

04-032

CHANGES IN PATENCY AND FOLLICULAR VITELLIN CONTENT IN *Tenebrio molitor* INFECTED WITH *HYMENOLEPIS DIMINUTA***T.J. Webb, K. Ellams and H. Hurd**

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Fecundity depletion in *Hymenolepis diminuta*-infected *Tenebrio molitor* is associated with a severe disruption in vitellogenesis, leading to decreased egg production and viability. Although the titre of juvenile hormone, which is known to coordinate these events, is unaltered, binding of the hormone to follicle cell sites is reduced. Thus, patency is delayed and the passage of vitellogenin to the oolemma is hindered. In an effort to determine whether these changes may be due to a juvenile hormone binding inhibitor, patency studies have been carried out at day 3 post-infection. The results suggest that the patency index of follicles from infected insects increases to control values after *in vitro* incubation (26°C; 6h; 3 changes of medium), and hence, the possible removal of inhibitory substances. ELISA detection of vitellin in 6 size ranges of follicles (0-1000µm in length) indicated that depreciations in yolk protein content are most marked in follicles of <200µm and early in the infection (day 3; 77.5% reduction): follicles appear to attain the correct length, but contain significantly less vitellin.

04-033

EFFECT OF THE ADULT FEEDING AND ENDOCRINE ON THE VITELLOGENESIS AND OVARY DEVELOPMENT IN THE ORIENTAL ARMYWORM *MYTHIMNA SEPERATA* WALKER (LEPIDOPTERA : NOCTUIDAE)**H.Gong, C.Cao**

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The yolk protein was prepared from the matured eggs by extraction with PBS buffer and subsequent precipitation in cold distilled water. The molecular weight of the native vitellin was determined as 205 Kd and the two subunits with molecular weights 142Kd, 47Kd by SDS-PAGE. The total protein contents and yolk protein titer of the hemolymph, fat body and ovary during the adult reproductive period was monitored by SDS-PAGE, rocket immuno-electrophoresis and protein assay.

In adult allatectomized within 24h after eclosion, the vitellogenin could not be detected in hemolymph and the ovary could not develop during the adult stage. JHA could restore the vitellogenesis and ovary development. The facts showed the JH served as a key factor to regulate vitellogenesis and ovary maturation during the reproductive stage. To test whether feeding behavior or nutrition effect on vitellogenesis and ovary development. It was exhibited that the initiation of the vitellogenin synthesis did not depend on the nutrition or feeding behavior, the activating of the CA was neither related to the nutrition during the adult stage nor the action of adult feeding, but adult feeding was required for the continual maturation to amount of eggs. So the effect of the nutrition on the vitellogenesis and ovary development may be through that nutrition promotes the endocrine activity.

04-034

YOLK PROTEIN DEGRADATION IN *HYALOPHORA CECROPIA* EMBRYOS.**E.C. Rubenstein, Y. Sharma, and A.A. Silver**

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Yolk proteins stored in developing insect oocytes are subsequently degraded during embryogenesis. In embryos of the giant silkworm *Hyalophora cecropia*, the yolk proteins are processed into smaller, immunoreactive peptides before complete breakdown. Degradation of the four principal yolk proteins is slow initially, but increases dramatically by day 11 of a 13 day developmental period. This is apparent in stained gels of a developmental series of embryonic extracts, as well as in Western blots for specific yolk proteins. Using zymography, we have identified several proteases that are not detectable in embryo homogenates earlier than day 11. The proteases detected differ with regard to specificity for the two substrates tested and optimum pH for detection. Auto-digestion studies of embryo homogenates at different pH values suggest that the major proteolytic activity responsible for the degradation of vitellin, the major yolk protein, is not present in the embryo until the last two days of development.

04-035

DEVELOPMENT OF THE WING BUDS IN *MEGOURA VICIAE* BUKT. (HOMOPTERA: APHIDIDAE).

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Previous investigations (Benedetti et al., 1991) have demonstrated that the wing buds in both males and females of *Megoura viciae* and *Acyrtosiphon pisum* are already present two days before birth. The morphological study was extended to parthenogenetic females and to males of *M. viciae* from birth (time 0) to IV instar.

In males, the wing bud epithelium is initially monostratified, becoming bistratified and then progressing to polystratified. Mitotic activity is more intense in the proximal bud, and its behaviour seems to synchronous and follows successive cycles.

TEM observations of the zone between the two buds, which in the adult will become two wings, show small cells lacking mitotic activity. Moreover, from birth to II instar, numerous vacuoles become evident in the wing epithelium.

In parthenogenetic females, which are presumably wingless, the buds resemble those of males from birth to II instar; subsequently, the wing epithelium returns to the monostratified state, and in the IV instar, it cannot be distinguished from the adjacent epithelium.

These observations, in addition to describing for the first time the initial phases of wing morphogenesis, define distinctive features of the wing bud that can be used to identify the presence or absence of these buds during development in other Aphid species.

04-037

A HORMONE FROM THE TSETSE FLY UTERUS STIMULATES PARTURITION AND ABORTION

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Unlike most insects, the tsetse female gives birth to a single, fully-grown larva at the culmination of each pregnancy cycle. The expulsion of the larva is regulated by a hormone present in rich abundance within the female's uterus. The hormone elicits parturition when injected into neck-ligated females at late stages of pregnancy and abortion when injected at earlier stages. We refer to this highly active material (0.043 uterus equivalents stimulates parturition in 50% of the females) as parturition hormone. Injection of the active extract, which appears to be a peptide or small protein, initiates the series of blood pressure pulsations and uterine contractions normally associated with parturition. The discovery that a uterus extract from the flesh fly also elicits parturition in tsetse suggests that this hormone may be widely distributed in insects.

04-036

THE RELATIONSHIP BETWEEN WING FORM AND FLIGHT MUSCLE DEVELOPMENT IN THE ORIENTAL CHINCH BUG *CAVELERIUS SACCHARIVORUS* OKAJIMA (HETEROPTERA, LYGAEIDAE)

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The relationship between wing form and flight muscle development was investigated for the adults of the oriental chinch bug, *Cavelerius saccharivorus*, which is an important pest of sugar cane in the subtropical region in Japan. As a result, it was found that there is a positive correlation between the relative wing length (RWL) and the % of retaining the fully developed flight muscles in both sexes. Almost all of the brachypters with RWL of 3.5-4.0 had undeveloped flight muscles, while a considerable portion of macropters with RWL more than 4.5 had fully developed flight muscles. The macropters with RWL of 4.5 were intermediate in the developmental degree of flight muscles. However, it should be noted here that some of the macropters did not have fully developed flight muscles. This indicates that some of the macropters of this species can not necessarily fly.

Based on the above results, the adults of *C. saccharivorus* were classified into three groups; adults with undeveloped wings and flight muscles, adults with developed wings and undeveloped flight muscles, and adults with developed wings and flight muscles. These phenomena were explained by the difference in the threshold level of response to any effectors such as JH between wing form determination and flight muscle form determination.

04-038

PHOTOPERIODISM AND THE ENDOCRINE ASPECTS OF ADULT DIAPAUSE IN *PROTOPHORMIA TERRAENOVAE*

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Protophormia terraenovae (Robineau-Desvoidy) (Diptera: Calliphoridae) showed a long-day photoperiodic response: Adults began to reproduce promptly after emergence under long-day conditions, whereas under short-day conditions they entered diapause without vitellogenesis. Temperature also showed a notable effect on the induction of diapause.

Removal of the corpus allatum (CA) inhibited ovarian development in nondiapaused adults. After application of a juvenile hormone analogue, implantation of the CA, or cutting of the nervi corporis allati in diapause adults, the adults fully developed their ovaries. Thus an inactivity of the CA to secrete juvenile hormone induces adult diapause in *P. terraenovae* and the CA activity is inhibited by the brain by way of the nervi corporis allati.

When the compound eyes were bilaterally covered by silver-containing paint, most of the flies entered diapause even under a long-day photoperiod or continuous light. These results show that *P. terraenovae* perceives photoperiod through its compound eyes.

04-039

ACTIVITY OF Mg^{2+} -DEPENDENT DEOXYRIBONUCLEASE IN ONTOGENESIS OF THE COLORADO POTATO BEETLE *LEPTINOTARSA DECEMLINEATA* SAY

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The activity of Mg^{2+} -dependent DNAase of the Colorado beetle was shown to depend on the morpho-functional state of the organism. The most marked changes in the dynamics of total activity of Mg^{2+} -dependent DNAase were noted in the physiologically active beetle, i.e. during the period of growth and development, metamorphosis, feeding and reproduction. The enhancement of the deoxyribonuclease reaction is characteristic before every larval moult, its highest intensity has been observed in the end of the IV instar. The least level of DNAase activity was found in eggs. The dynamics of total activity of Mg^{2+} -dependent DNAase no marked changes in the state of winter rest (winter diapause, oligopause and spring res-toration period).

04-041

ARTIFICIAL DIETS FOR CITRUS-FEEDING SWALLOWTAIL BUTTERFLIES (LEPIDOPTERA: PAPILIONIDAE)

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Citrus-feeding swallowtail butterflies, Genus *Papilio*, have been utilized as materials for various experimental purposes in the biological sciences as well as exhibits in museums or insectariums. We developed artificial diets for the butterflies by modifying those for the silkworm, *Bombyx mori*, for the use of the insects as experimental animals throughout the year.

The artificial diets contained 50% of leaf powder of *Fagara arisanthoides*, on a dry weight basis. The fresh leaves were dried by employing three different procedures; (1) freeze drying, (2) drying at 90°C completely, and (3) drying at 90°C after steaming for 5 minutes. *Papilio xuthus*, *P. protenor*, *P. polytes* were used for the experiment. Each ten newly hatched larvae were confined in a plastic cup (10ml) with small amount of the diet. Eggs, larvae and pupae were maintained at 25±2°C, 50~60% RH, and under a long day photoperiod(16L:8D).

It was evident that the drying condition influences largely the food values of *Fagara arisanthoides* leaves. Relatively good growth was obtained in all the species on the diets containing the leaf powder dried after steaming, while a very poor growth was obtained on those containing the leaf powder dried without steaming or freeze-dried. The mortality was very high during the first instar larval stage compared to that during the other instar larval stage. We observed that the decrease of survival during the stage from the 2nd to last instars was mainly caused by devouring. The weight of the pupa and size of the adults were almost the same as those of insect reared on fresh leaves. We also confirmed the fertility of the butterflies reared on these artificial diets. Some other species belonging to Genus *Papilio* also could be reared on these artificial diets, although their fertility has not been confirmed.

04-040

NUTRITIONAL INDICES OF SECOND INSTAR LARVAE OF *PLUTELLA XYLOSTELLA* L. FED UPON LEAVES TREATED WITH EXTRACT FRACTIONS AND ISOLATES OF *MELIA AZEDARACH* L.

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Nutritional indices like relative growth rate (RGR), efficiencies of conversion of ingested food (ECI) and conversion of digested food (ECD) showed a linear decreasing trend over solvent check, on increasing concentration from 0.0125 to 0.05% of chloroform : methanol (9:1) crude extract of *Melia azedarach* L. and its hexane, diethylether, ethyl acetate and chloroform fractions against second instar larvae of *Plutella xylostella* L. After four days of exposure, significantly less food was consumed and larval weight gained was lower by 1.5 times. The relative consumption rate (RCR) remained unaffected except in chloroform and crude extract (both @ 0.05%). In hexane, chloroform and crude extract, ECI and ECD indices were reduced to their lowest and ranged from 40.3 to 43.6% and 41.7 to 45.2%, respectively as compared with other treatments (ECI = 48.7 to 48.9%; ECD = 50.8 to 52.2%) and the solvent check (ECI = 56.9 to 60.0; ECD = 58.3 to 61.6%).

In gedunin and ochinolid-B treatments, food consumed and weight gained by the larvae was greatly reduced. RGR was reduced from 0.56 in solvent check to 0.39 in gedunin and 0.40 in ochinolid-B (both @ 0.005%). ECI and ECD indices were also significantly reduced in these treatments.

04-042

SOLID ARTIFICIAL DIET WITH TOMATO JUICE FOR LARVAE OF *BACTROCERA OLEAE* (DIPTERA: TEPHRITIDAE)E. I. Navrozidis¹ and M. E. Tzanakakis²

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Larvae of *Bactrocera oleae* (Gmelin) (Diptera, Tephritidae) were reared on a reference artificial diet P, in which the water was replaced by canned tomato juice, and the nutritive components were gradually reduced or omitted altogether one by one.

The diet finally selected was without olive oil, Tween-80 and hydrolysed protein, and consisted of: tomato juice 55 ml, cellulose powder 26 g, brewer's 7.5 g, roasted peanuts 3 g, potassium sorbate 0.05 g, methyl-p-hydroxybenzoate 0.2 g and HCl 2N 2 ml to a pH of 4.

The yield of this simpler diet in adults over neonate larvae was 73.8 %, the mean pupal weight 7.1 mg and larval development was completed in 9.5 days at 25±1°C. The cost of ingredients was approximately 15% lower than that of the reference diet P.

04-043

SXL HOMOLOGUE IN *CERATITIS CAPITATA*: *CcSXL* TRANSGENIC *DROSOPHILAS* AS A TOOL TO STUDY THE CONSERVATION OF FUNCTIONAL DOMAINS.

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The molecular bases of sex determination in dipterans are widely unknown. However, it is possible that a common principle exists, according to which different primary signals control the activity of a binary switch gene (Nöthiger and Steinmann-Zwicky, 1985, Cold Spring Harbor Symp. Quant. Biol. 50: 615-621).

In *Drosophila*, *Sex-lethal* (*Sxl*) is the master regulator gene responding to the X:A primary signal and its activity leads to female development. To ascertain whether in *C. capitata* a similar sex-differentiation molecular pathway was employed in spite of the different sex-determining primary signals, we underwent the study of the evolutionary conservation of the *Sxl* gene in this organism.

On the basis of the analysis of several *C. capitata* cDNA clones we established that the *Sxl* gene is highly conserved at the nucleotidic sequence level. However, *CcSxl* is not sex-specifically expressed. To test whether the ectopic expression of *CcSxl* in *Drosophila* males was able to induce sex-specific lethality or sexual transformation, *CcSxl* cDNAs were placed under the control of the *hsp70* promoter and introduced into *Drosophila* germline by *P* element-mediated transformation. A collection of 0-12 h transgenic embryos was heat-shocked and stained with anti-SXL antibody, to check the expression of CcSXL protein. At the phenotypic level, we observed an abnormal differentiation of external male-specific structures in different *Drosophila* transgenic lines, suggesting that CcSXL protein can interfere to some extent with the sex-specific splicing of the downstream sex-differentiation gene (*transformer*). Further studies are currently underway to investigate the effect of the ectopic expression of *CcSxl* in different *Sxl* mutant backgrounds.

04-045

EFFECTS OF WING AMPUTATION, BODY SCORCHING, AND PREVENTION OF FLIGHT ON OVARIAN MATURATION IN THE OLIVE FRUIT FLY *BACTROCERA* (*DACUS*) *OLEAE* (DIPTERA: TEPHRITIDAE)

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Ovarian maturation is prevented in *Bactrocera* (*Dacus*) *oleae* (Gmelin) developed in the pre-imaginal stages at LD 12:12, 19±1°C and kept as adults at LD 16:8, 26±1°C. When females developed and maintained under these conditions had their wings amputated or cut off at half their length, or their thoracic notum cauterized, at an early age, a high percent of them had mature oocytes one or two weeks after treatment. Cutting off the halteres, which did not prevent flying, resulted in an intermediate effect between the females with amputated wings and those of the control. The younger the females at treatment, the stronger the favorable effect on ovarian maturation. Females with their wings amputated, laid more eggs in olive fruit during their first 3 weeks than untreated females. Intact females kept during their third week in narrow vials where flying was not possible, soon matured their oocytes in contrast to the control.

04-044

PROSTAGLANDIN E2 IN THE SILKMOTH, *BOMBYX MORI*, WITH SPECIAL REFERENCE TO THE STIMULATION OF EGG-LAYING

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Investigation of the role of prostaglandins (PGs) in the reproductive behaviour of *Bombyx* revealed that PGs play no crucial role as oviposition behaviour stimulants. Injection of prostaglandin E2 (PGE2) had no effect on the egg-laying behaviour of virgin females. Oviposition behavior of females injected with PG biosynthesis inhibitors such as indomethacin, quinacrine and NDGA was not interrupted or suppressed. Sterilized males of *Bombyx* can be induced by heat treatment (32°C) for 72 hrs during their wandering stage. The rate of oviposition of female moths mated with sterilized males was quite suppressed, compared to the control, in the 24 hrs ensuing mating. PGE2 injection on the females mated with these sterilized males had no effect on restoring oviposition behaviour. There were no significant differences in the PGE2 content of testes between normal and sterilized males. After mating, the amount of PGE2 increased with either normal or sterilized males. These results indicate that PGs do not seem to be directly involved in the egg-laying behaviour of *Bombyx*, although PGs may have some functions in the reproduction of this insect.

04-046

REPRODUCTION AND SURVIVAL OF ADULT *DACUS* (*BACTROCERA*) *OLEAE* (GMELIN) (DIPTERA: TEPHRITIDAE) AFFECTED BY DIETARY ADMINISTRATION OF ANTIAMINOACIDS

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From a series of 30 anti-amino acids, added individually to the adult diet of *Dacus* (*Bactrocera*) *oleae*, the following ten were significantly shortened the life span of both sexes: L-canavanine (arg), 3,4-Dihydro-DL-proline (pro), L-glutamic acid-γ-hydrazide (gln), D-cycloserine (ala), L-methionine sulfoximine (met) and DL-p-fluorophenylalanine (phe). The parentheses indicate the antagonized normal amino acids. In low concentrations these substances reduced the fecundity, without any significant decrease in hatchability.

Female fecundity and egg hatchability were significantly affected, up to zero, by the following three anti-amino acids: 4-amino-DL(L)-phenylalanine (phe), Allyl-glycine (cys) and 3-amino-L-tyrosine (tyr).

04-047

THE MALE REPRODUCTIVE SYSTEM OF *TENEBRIO MOLITOR* IS AFFECTED BY THE PRESENCE OF *HYMENOLEPIS DIMINUTA* (CESTODA) METACESTODES.

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Hymenolepis diminuta is known to adversely affect the reproduction of female intermediate hosts. This is the first study of the effects on male reproduction. Initial studies were focused on the bean-shaped accessory glands (BAGs). The size, weight, trehalase activity, protein content and protein profiles of glands from infected and uninfected beetles were compared at different days post-emergence. BAGs from infected males continued to increase in size and weight from 6-10 days post-emergence, when uninfected glands had ceased growing. Trehalase activity reflected the change in size by showing an increase in activity per whole gland. Protein profiles of infected glands remained unchanged but total protein content per gland was significantly increased.

More recent studies have been concerned with the effect of the parasite on the testes and spermatozoa of *T. molitor*. Sperm motility and oxygen consumption / CO₂ production were assessed and results will be discussed with respect to a possible enhancement of male reproductive potential.

04-049

EXPRESSION OF HUNCHBACK, KRÜPPEL AND EVEN-SKIPPED IN THE LOWER DIPTERAN *CLOGMIA ALBIPUNCTATA* WILLISTON (NEMATOCERA: PSYCHODIDAE)

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The mothmidge *Clogmia albipunctata* is considered to be a primitive nematoceran and shows a morphological long germ development with some differences to *Drosophila melanogaster*.

As the comparison of gene expression has become an useful approach to get information about the evolution of developmental mechanisms, we searched for the segmentation gene (gap gene) orthologues hunchback and Krüppel by PCR in *Clogmia* and used the PCR products as probes for in situ hybridizations. In addition, we used an interspecifically reacting monoclonal antibody to visualize the protein expression of the segmentation gene (pair rule gene) orthologue even-skipped in *Clogmia* embryos.

The expression patterns of the segmentation gene orthologues hunchback, Krüppel and even-skipped show some differences to *Drosophila*. In the late appearance of some posterior elements of the patterns, *Clogmia* seems to have some aspects of short germ development. Differences of the posterior domains of hunchback and Krüppel expression could reflect a different mode in the generation of the most posterior segments.

In general, secondary expression is more variable than primary expression, as it is already known from other species. We suggest that the basic functions of these genes are conserved between *Drosophila* and *Clogmia*. However it is surprising that secondary expression patterns differ in such a striking way between closely related species. Most striking is the complete absence of a secondary set of even-skipped expression in *Clogmia*.

04-048

OVARIAN DEFECTS IN HYBRIDS OF THE SPECIES PAIR *DROSOPHILA VIRILIS* AND *DROSOPHILA TEXANA*
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In interspecific matings between *Drosophila virilis* and *Drosophila texana* female sterility is observed in F₂ hybrid females. A previous study has shown that no vitellogenin synthesis occurs in the fat body of sterile hybrid females. The results of this work show that hybrid ovaries of sterile females transplanted into the abdomens of females of the parental species are not able to develop upon maturity. With few exceptions, the hybrid ovaries remained alive in the host environment, but their oocytes failed to develop to vitellogenic stages.

Thus, in hybrid females between *Drosophila virilis* and *Drosophila texana* sterility is the result of defects in at least two developmental processes, synthesis of vitellogenins in the fat body and uptake of vitellogenins by the developing oocytes.

04-050

ISOLATION AND CHARACTERIZATION OF A *BOMBYX VASA*-LIKE CDNA

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As a first step toward the establishment of germ cell culture system in the silkworm, *Bombyx mori*, we attempted to obtain a molecular marker of the germ cell. DEAD BOX family includes many genes that are preferentially expressed in germ line of a wide range of animal species and also the family protein has many evolutionary conserved amino acid sequence motifs useful for the isolation of unknown family genes by PCR. Here, we searched for DEAD BOX family genes expressed in *Bombyx* ovary by RT-PCR using degenerate oligonucleotide primers which correspond to such motifs. We obtained five cDNA species belonging to the family. Of those, a cDNA clone showed considerable similarity in the deduced amino acid sequence to that of the *Drosophila vasa* gene product. Using this clone, a cDNA clone containing the full ORF was isolated from a *Bombyx* ovary cDNA library. The sequence of the clone revealed that a N-terminal part of the amino acid sequence contains glycine rich region. Northern analysis showed that the mRNA existed in the egg, suggesting that it functions as a maternal factor. All these characteristics are shared with *Drosophila vasa*. An antibody directed against a bacterially synthesized polypeptide of the cDNA was made and the function of the gene in the early development is now under investigation.

04-051

DEVELOPMENTAL ANALYSIS OF LEG AND GENITALIA FORMATION OF THE SILKWORM, *BOMBYX MORI*, USING GYNANDROMORPH MUTANT

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In this study, a hybrid race of the silkworm, *w^{PSa}mo, p, w-2/+ , mln/+ X mo, p(or ps/p), w-2, mln*, was used. This hybrid mutant produces gynandromorph mosaic in a high rate.

In early embryological studies, it was observed that larval legs were formed as extrusion of ventral part of the integument. However, individuals with pleurite/sternite dissociation of larval body color imply that legs are produced first as an extrusion of both pleurite and sternite to lateral direction, and then move downwards, because cells of the pleurite come down to tip of the legs making a wedge shape in the sternite background.

In the median longitudinal dissociation, female and male genitalias are formed independently in one side and the other. However, in some cases, labia and ancus are produced bilaterally. These facts mean that imaginal discs of labia and ancus possibly have a potency of bilateral regeneration.

04-053

MOLECULAR CLONING AND GENOMIC ORGANISATION OF A NOVEL RECEPTOR FROM *DROSOPHILA MELANOGASTER* STRUCTURALLY RELATED TO MEMBERS OF THE GLYCOPROTEIN HORMONE (THYROID STIMULATING HORMONE, FOLLICLE STIMULATING HORMONE, LUTEINIZING HORMONE, CHORIOGONADOTROPIN) RECEPTOR FAMILY FROM MAMMALS

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In mammals, reproduction is controlled by: (1) steroid hormones, (2) a family of closely related glycoprotein hormones produced by the adenohypophysis (luteinizing hormone, LH; follicle stimulating hormone, FSH), or placenta (choriogonadotropin, CG), and (3) hypothalamic releasing hormones (e.g. luteinizing hormone releasing hormone, LHRH) that control the release of LH and FSH from the adenohypophysis. Several years ago, the receptors for mammalian FSH, LH and CG have been cloned, as well as the receptor for a fourth glycoprotein hormone, thyroid stimulating hormone (TSH) that is also produced by the adenohypophysis. The receptors for LH and CG appeared to be identical. All three receptors are closely related and form a subfamily belonging to the large family of G-protein-coupled (7 transmembrane) receptors. Using oligonucleotide probes derived from consensus sequences for these glycoprotein hormone receptors, we have now cloned a 831 amino acid residues long receptor from *Drosophila melanogaster* that shows a striking structural homology with the TSH, FSH, LH/CG receptor family from mammals. This homology includes a very large, extracellular N terminus (20% sequence identity with the rat TSH, FSH, LH/CG receptors) and a 7 transmembrane region (53% sequence identity with the rat receptors). The *Drosophila* receptor gene is >7,5 kb long and contains 17 exons and 16 introns. Many of the intron-exon transitions coincide with the intron-exon transitions of the mammalian glycoprotein hormone receptor genes. All this indicates that the *Drosophila* receptor is evolutionarily related to the mammalian receptors. We expect that the novel insect receptor is involved in insect reproduction or development.

04-052

LOCALIZATION OF LOCUSTATACHYKININ-I-IMMUNOREACTIVE PEPTIDES IN DEVELOPING EMBRYO OF *DROSOPHILA MELANOGASTER*

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Drosophila embryos were described to produce various kinds of peptides during their developmental stages. We confirmed that, in *Drosophila melanogaster*, one of them is Locustatachykinin I, which has been purified in brain and retrocerebral complex (corpus cardiacum-corpus allatum), and has been also shown to play major roles as a myotropic contraction in foregut and oviducts in *Locusta*. The locustatachykinin-I-immunoreactive (LomTK-I-IR) peptides are first seen as several stained spots in both anterior and posterior tips during developmental stages of *Drosophila* embryo. This stage is thought to be early stage of neurulation. In the mid stage of neurulation, LomTK-I-IR peptides are found mostly in medial dorsal region of posterior part of *Drosophila* embryo, as well as in anterior and posterior regions. After the neurulation, the LomTK-I-IR peptides are located as one or two large staining clumps of the embryo. Thereafter, the LomTK-I-IR peptides are strongly stained in three segments of thoracico-anteroabdominal part of the embryo. When the embryo development is nearing along toward 1st instar larval stage, the localization pattern of the LomTK-I-IR peptides differs from that localized in earlier embryo. The three large clumps of the LomTK-I-IR peptides are changed first into various small spots, and then into more large spots, especially in ventral regions of the embryos. Thus, the embryo nearer to 1st instar larva shows various large LomTK-I-IR spots in ventral portion of the embryos.

04-054

TRANSCRIPTIONAL REGULATION OF A CHORION GENE PAIR DURING OVARIAN DEVELOPMENT IN THE SILKMOTH *BOMBYX MORI*.

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The chorion of the silkworm, *Bombyx mori*, is composed of over 100 different proteins that are synthesized and secreted in a specific order during oogenesis by follicular cells surrounding the developing oocyte. Chorion protein synthesis is regulated at the transcriptional level. Chorion genes are organized in divergently oriented pairs separated by short, common promoter regions containing all the elements required for normal expression.

Research in our laboratory focuses on the establishment and implementation of the choriogenic developmental program in follicular cells. We have previously identified two proteins, BCFI and BCFII, that bind specifically to the promoter of HcA/B.12, a chorion gene pair exclusively expressed during the late stages of choriogenesis and used as a model in our studies. Although BCFI only appears in the nuclei of follicular cells at the late stages of choriogenesis, coincident with the expression of HcA/B.12, strong evidence suggests that it is actually synthesized much earlier and stored in the cytoplasm in a phosphorylated form as an inactive precursor. BCFI binds to the DNA sequence AGATAA on the HcA/B.12 promoter and is a product of the gene BmGATA β , which encodes at least 3 isoforms by alternative splicing. The 3 proteins encoded by BmGATA β belong to the GATA family of zinc finger-containing DNA-binding proteins, and differ from one another in the organization of their DNA-binding domains.

Our present aim is to determine how the three GATA proteins encoded by BmGATA β differ functionally. We are currently raising and characterizing isoform-specific antibodies that will be used to determine the abundance and subcellular localization of each protein isoform in follicular cells at different stages. The DNA-binding specificity of the three GATA proteins will be studied, and finally, their potential as transcriptional activators of the HcA/B.12 gene will be addressed using *in vitro* transcription assays.

04-055

THE SILKMOTH HOMOLOG OF THE VERTEBRATE NUCLEAR RECEPTOR HNF-4 : CLONING AND ANALYSIS OF EXPRESSION DURING FOLLICULAR DEVELOPMENT

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A cDNA library of follicular cells from vitellogenic follicles of *Bombyx mori* was screened under low stringency conditions with a probe derived from the DNA binding domain of the ecdysone receptor for the presence of additional members of the steroid hormone receptor superfamily. The predicted amino acid sequences of seven isolated clones are identical in their DNA binding, hinge and ligand-binding domains, but could be grouped into two classes with respect to their amino-termini (activation domains). All clones share close similarity with the vertebrate orphan receptor hepatic nuclear factor-4 (HNF-4) in their DNA binding domain (88 % at the amino-acid level).

Southern blot analysis shows that the silkmouth HNF-4 homolog (BmHNF-4) is present as a single copy gene in the genome. Northern blot analysis detects two mRNA species of 4.8 and 5.2 kb that are predominantly present in follicular/nurse cells of maturing follicles. The mRNAs accumulate in the oocyte during vitellogenesis and are abundant in newly-laid eggs. The RNA expression patterns therefore suggest that the BmHNF-4 mRNAs are maternal mRNAs that play a role in early embryonic development. Upon *in vitro* expression, BmHNF-4 shows strong binding to the vertebrate HNF-4 DNA-binding site of the apolipoprotein CIII promoter. A similar binding activity could also be detected in nuclear extracts prepared from vitellogenic follicles, suggesting that BmHNF-4 also functions as a transcription factor in the nuclei of follicular cells.

04-057

EFFECT OF Ca^{++} ON THE CATHEPSIN B-LIKE PROTEINASE IN TUSSAH, *ANTHERAEA PERNYI*

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To clarify the mechanism of the activation of the egg proteinases was significant for the controlling of pest insects. Although several kinds of egg proteinases have been studied and purified from some insects, the mechanism of the activation about the egg proteinases has not been completely clarified up to now.

The effects of the metal ions on the cathepsin B-like proteinase in *Antheraea pernyi* were studied by using the purified proteinase. The results showed Ca^{++} could increase the activity of the proteinase. The bovine serum albumin and vitellin could be well hydrolyzed by the cathepsin B-like proteinase when the Ca^{++} was existing, but not when the Ca^{++} was absent. The results suggested that Ca^{++} stimulation might be one of the mechanism of the activation in this egg proteinases.

04-056

STUDIES ON THE PROPERTIES OF THE EGG PROTEINASES IN COTTON BOLLWORM, *HELIOPERPA ARMIGERA*

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A proteolytic activity was detected in the developed oocytes of *Helicoverpa armigera*. The optimum pH for the enzyme was 3-5. The activity of the enzyme could be inhibited by N-[N-(1,3-trans-carboxyoxiran-2-carbonyl)-L-leucyl]-agmatine (E-64), pepstatin and diisopropyl fluorophosphate (iPr₂P-F). A highest hydrolysis rate was detected at pH 4 when the bovine hemoglobin was used as the substrate. The antisera to the cysteine proteinase and aspartic proteinase of *Philosamia cynthia ricini* could recognize the antigene in the oocytes of *H. armigera*. The results suggested that there were cysteine proteinase and aspartic proteinase in the oocytes of *H. armigera*, and the properties of the proteinases were similar to those in *P. C. ricini*. Moreover, the vitellin from *H. armigera* was identified and compared with that in *P. C. ricini*.

04-058

HORMONAL REGULATION OF VITELLOGENESIS IN THE SOFT TICK, *ORNITHODOROS MOUBATA* (ACARI: ARGASIDAE)

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We determined that juvenile hormone (JH) does not regulate vitellogenin (Vg) synthesis in *Ornithodoros moubata*. Therefore, we undertook studies to determine the hormonal regulation of vitellogenesis in adult females of this species. Normal induction of vitellogenesis requires mating and engorgement. However, we were able to stimulate vitellogenesis in unfed mated females by injection with synganglion (SyG) extracts and topical application of cypermethrin. Ligation experiments revealed that Vg synthesis is regulated by two factors. First, vitellogenesis inducing factor (VIF) is released from the SyG. VIF in turn stimulates a tissue in the posterior portion to release a second factor, fat body stimulating factor (FSF), which stimulates the fat body to synthesize Vg. Experiments indicate that VIF is a peptide and FSF may be an ecdysteroid. Further studies showed that ecdysteroid titres in the hemolymph increase after feeding and injections of ecdysteroids stimulate low levels of Vg synthesis in unfed mated females.

04-059

EXPRESSION OF AUTOGENY DEPENDING ON TENERAL RESERVES IN CULEX AND ANOPHELES (DIPTERA: CULICIDAE)

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Total protein and lipids present at eclosion of teneral female mosquitoes largely reflect the larval feeding history. In addition, the genotype determines the metabolic potential for biosynthetic processes, particularly of total protein and reserve lipids. Most clearly this is expressed when comparing autogenous strains of mosquitoes that are able to accumulate more reserves during their larval development with their anautogenous conspecifics. The excessive protein contents in autogenous genotypes allow the maturation of their first batch of eggs without blood meals.

Our previous studies revealed minimal amounts of total protein and lipids in teneral Anophelines when compared to Culicines. This became particularly evident when the caloric measurements were normalized for a constant body size. Based on these findings it appeared unlikely for Anophelines to evolve autogeny as a mode of reproduction. Therefore it was interesting to explore the situation in the autogenous species *Anopheles near salbaii* (kindly provided by Dr. A. Spielman). As controls we chose *An. gambiae*, an anautogenous representative of similar body size and, furthermore we reinvestigated autogeny in *Cx. pipiens*.

The autogenous *An. nr. salbaii* had twice the protein content per female at eclosion than the anautogenous *An. gambiae*, while its lipid content was even quadrupled. In *Cx. pipiens* there were similar trends but much weaker in extent (protein increased by 20%, lipid doubled). Furthermore, all these proportions showed positive correlations with body size. After oviposition, female protein and lipid contents were equal for autogenous and anautogenous phenotypes, but species-specific.

Detailed information on the kinetics of yolk deposition, its subsequent partial resorption, and the maternal investment during oogenesis will be presented and discussed for the various phenotypes studied.

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04-061

FIRST RECORD OF GYNANDROMORPHISM FOR ANACRIDIDIUM AEGYPTIUM (L.) (ORTHOPTERA: CATANTOPIDAE)

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Gynandromorphism has been scarcely recorded among Orthoptera in comparison with other orders of Insects, such as Lepidoptera and Hymenoptera. As far as Ensifera are concerned, the evidence of this rare condition is more immediate, whereas it is morphologically less marked among Caelifera, according to slighter external differences between the sexes; the described gynandromorphs amount at present to a few dozens and refer to about twenty-five species for the last suborder. The study of this phenomenon involves several branches of general entomology, such as genetics, embryology, external morphology, anatomy, ethology, and it is interesting also as regards the taxon concerned. A bilateral gynandromorph of *Anacrididium aegyptium* (L.) (Orthoptera Caelifera: Catantopidae) was collected near Imperia (Northern Italy) in February 1993. Since the specimen came already dead under observation, behavioural data are not available: the external morphology, which exhibits various asymmetries, could be only considered. The size of the specimen is approximately intermediate between the mean male and female dimensions for this species. In the head the right antenna is slightly longer than the left. In the thorax a small distortion is evident in the prothorax, the right paranotum being a little longer than the left, and the metathoracic pair of legs is asymmetrical, the right leg being slightly longer. In the genital and post-genital segments of the abdomen there is lateral bipartition of the external morphological characters which are female on the right side and male on the left, as in the majority of gynandromorphs described up to now for Orthoptera. This is evident in the VIII sternite, which forms the right half of the female subgenital plate, and in the X tergite, epiproct, paraprocts, and cerci. The left half of the male IX sternite is present and prolonged in the left half of the male subgenital plate. On the right side, the two ovipositor valves are present: the dorsal valve is normal in shape, while the ventral is slightly anomalous and deviated medially. No instance of gynandromorphism results previously reported for this species.

04-060

ULTRASTRUCTURAL PROPERTIES OF THE FOLLICULAR EPITHELIUM OF THE HONEY BEE APIS MELLIFERA (L.) DURING VITELLOGENESIS

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During previtellogenesis the follicular epithelium is tightly closed when the cells are connected with each other laterally by continuous *Zonulae adherentes*. During vitellogenesis the follicle cells show a hexagonal pattern in horizontally orientated sections. The *Zonulae adherentes* are still present almost all around each cell, but tiny, pore-like intercellular canals appear in the corners where always three cells meet. These canals connect the intercellular space basally of the follicle cells with the perivitelline space between follicular epithelium and oocyte. In the medial part of each canal a F-actin bundle is found closely inside each of the three participating cells stretching horizontally from one cell wall to the other. Together these three bundles form a ring around the canal. From these rings F-actin bundles stretch horizontally along the tight connected lateral cell membranes. Apically the follicle cells extrude long, finger-like protuberances through the narrow perivitelline space and deep into the oocyte. They are densely filled with longitudinally arranged F-actin bundles. The lumen of the canals apically of the F-actin rings and the perivitelline space are filled with an electron dense substance which contains vitellogenin; basally of the rings the lumen of the canals and the intercellular space are devoid of this substance. Canals, protuberances, and their F-actin elements may be involved to direct the vitellogenin towards the surface of the oocyte where it is taken up by endocytosis.

04-062

THE INFLUENCE OF FEEDING AMOUNT ON THE DEVELOPMENT DURATION OF MYRMELEON (MORTER) SAGAX LARVA

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Myrmeleon sagax (Neuroptera: Myrmeleonidae) is one generations a year in nature condition. In laboratory condition, however, the feeding amount of larva can affect its development. Three generations in one year were observed in our experiment by providing sufficient food; one complete generation was observed from June to August, under the long photostage and high temperature conditions. It was found that the third instar larva differentiate after autumn by feed once in a interval of 15 days, a few larva can complete its development into adult after few months, and most of larva delay its development to next year.
Key words: ant-lion *Myrmeleon sagax*

Section 5

Cell Biology, Physiology and Biochemistry

05-002

POLYDNAVIRUSES AND PARASITOID HOST RANGE: AN IMMUNOLOGICAL VIEWPOINT

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Endoparasitic wasps of the families Braconidae and Ichneumonidae have developed a unique strategy to protect their developing progeny from the action of their host's immune system. The female wasps of these two families carry within their ovaries a virus which belongs to the family Polydnviridae. Typically, these viruses are injected along with the wasp's egg directly into the hemocoel of a larval host. Polydnviruses then, either alone or in concert with other wasp derived factors, induce profound changes in the cellular and humoral immune system of the parasitized host. The extent to which polydnviruses determine their parasitoid's host range is not known. We chose to use *Microplitis demolitor* and its associated virus, *Microplitis demolitor* polydnvirus (MdPDV) to address this question since previous work has shown that, in this particular system, persistence and expression of the polydnvirus is sufficient and necessary to cause immune disruption. Investigation of wasp progeny survival, immune function, viral persistence and expression was carried out using *Microplitis demolitor*, and three lepidopteran species as wasp hosts. These included *Pseudoplusia includens*, *Spodoptera frugiperda* and *Trichoplusia ni*. Preliminary studies indicated that these noctuid species varied in their permissiveness as wasp host. Results obtained to date suggest that persistence of polydnvirus expression is one of the key factors in determining whether or not a particular lepidopteran species is a suitable host for the wasp and support the hypothesis that polydnviruses act as true host range determinant.

05-001

DEVELOPMENTAL PROFILE AND CYTOPATHIC EFFECTS OF AN ENTOMOPOXVIRUS FROM A PARASITIC WASP

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Entomopoxvirus (DIEPV) particles from the venom apparatus (accessory gland) of the parasitic wasp *Diachasmimorpha (Bioesteres) longicaudata*, are introduced into Caribbean fruit fly (*Anastrepha suspensa*) larvae by ovipositing wasps. Mouse polyclonal antibodies generated against sucrose density gradient purified DIEPV detected viral proteins in Western blots of host hemocytes and to a lesser extent of host fat body <24 h post-parasitization. The intensity of the viral protein signal from host cells increased significantly by 36 h post-parasitization, coincident with the onset of the parasite's first larval stadium. Southern hybridization using digoxigenin-labeled DIEPV DNA as a probe, confirmed the presence of the virus within the hemocytes. Immunofluorescent labeling of host hemocytes using the anti-DIEPV protein serum and an FITC-labeled second antibody, localized viral proteins in hemocyte cytoplasm. The affinity of selected FITC-labeled lectins to virus-infected hemocytes was different from that observed for healthy hemocytes. Furthermore, DIEPV infection altered f-actin arrangement in infected hemocytes.

05-003

CYSTEINE-RICH PROTEIN FROM *CAMPOLETIS SONORENSIS* AND ITS ASSOCIATED POLYDNAVIRUS.

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Parasitoids employ polydnviruses, venoms and ovarian proteins to inhibit the immune system of their hosts during endoparasite development. In the *Campoletis sonorensis*-*Heliothis virescens* system, ovarian proteins acutely suppress the immune system at early times after parasitization while proteins expressed from the polydnvirus are required for chronic suppression. An ovarian protein with multiple glycoforms that is encoded by a single copy *C. sonorensis* gene and a family of cysteine-rich polydnvirus proteins appear to suppress the insect cellular immune response. Our studies have focused on the *C. sonorensis* polydnvirus VHv1.1 and VHv1.4 genes both of which are glycosylated and contain 2 copies of a motif with 6 cysteine residues each. The ovarian protein also is glycosylated and cysteine-rich (~3% cysteine). Although cysteine residues in the ovarian protein are clustered they do not comprise a motif similar to the polydnvirus motifs. We have begun a series of experiments to determine the functionally important regions of the parasitoid immunosuppressive proteins. These proteins bind and localize to hemocytes with internalization blocked by incubation at 4° C suggesting that this is an active process involving endocytosis of receptor protein complexes from the hemocyte surface. Cysteine residues are involved in disulfide formation for intramolecular linkage and confer structural stability necessary for biological activity. Treatment of cys-proteins with reducing agents which disrupt disulfide bridges within proteins, inhibits the binding of these proteins to hemocytes. As dithiothreitol concentrations increase, the binding of the proteins to hemocytes is significantly reduced. These genes have been expressed in the baculovirus system and linked to a poly-histidine tract allowing purification of individual proteins for functional analyses. As an initial step towards determination of the 3-dimensional structure the pattern of linkages within the cysteine motifs is being mapped. The importance of the carbohydrate residues as related to biological activity is also being evaluated by endoglycosidase-H digestion and by site-directed mutagenesis to remove or prevent glycosylation. Differences and similarities of the ovarian and polydnvirus proteins in terms of their expression, tissue-specific binding and effects on the *Heliothis virescens* immune system will be discussed.

05-004

IMMUNITY INTERACTIONS BETWEEN THE BRACONID *ASOBARA TABIDA* AND *DROSOPHILA* LARVAL HOSTS

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Avoidance of encapsulation by *Asobara tabida* (Hym.; Braconidae) in *Drosophila* larval hosts is the result of a physiological race between the host's ability to quickly form a hemocyte capsule and the aptitude of the parasitoid to get protected from the attack by the hemocytes. *Drosophila* ability to encapsulate *A. tabida* is associated with high number of circulating hemocytes. This association is verified among several *Drosophila* species of the subgroup *melanogaster*.

Avoidance of encapsulation by *A. tabida* is associated with a quick embedment of the parasitic egg within the host tissues. Recognition of the parasite by the host cells thus must be affected in such manner that host tissues (digestive tube, fat body, muscles and trachea) are strongly sticking to the parasite's egg chorion while the host hemocytes are not attaching to it.

No VLP were found in the accessory glands of the parasite. Factors responsible for the inhibition of encapsulation are investigated at both physiological and molecular levels.

05-006

MANIPULATION OF HOST (*SPODOPTERA LITTORALIS*) DEVELOPMENT AND ENDOCRINOLOGY BY THE PARASITOID *CHELONUS INANITUS*: EFFECTS OF POLYDNAVIRUS/VENOM AND OF THE PARASITOID LARVA

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Parasitization by the egg-larval parasitoid *Chelonus inanitus* strongly influences later stages of host development. Non-parasitized larvae pass through 6 larval instars, but parasitized larvae enter metamorphosis in the 5th instar. The parasitoid larva then emerges from the precocious prepupa and feeds on it. If the parasitoid fails to emerge, the host never pupates but remains developmentally arrested in the prepupal stage. We could show that polydnavirus/venom are responsible for induction of developmental arrest in the prepupa while the parasitoid larva plays a key role in inducing precocious onset of metamorphosis. We made comparative measurements of juvenile hormone (JH) biosynthesis in vitro, JH titres, ecdysteroids titres and juvenile hormone esterases in non-parasitized, parasitized or polydnavirus/venom containing larvae. Our data indicate that the presence of a late 1st instar parasitoid larva reduces JH biosynthesis by host c. allata and JH titers; this then induces the subsequent premetamorphic changes in JH esterase and ecdysteroids. In polydnavirus/venom containing larvae endocrine differences to non-parasitized larva become manifest only in the 6th instar, the main difference being modified JH esterase and ecdysteroid patterns.

05-005

LIFE CYCLE AND FUNCTIONS OF THE POLYDNAVIRUS OF THE BRACONID WASP *CHELONUS INANITUS*

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The calyx cells of the ovary of *C. inanitus* contain large numbers of polydnaviruses (PDV) (Albrecht et al., 1994, J. Gen. Virol. 75, 3353-3363). We could show that viral DNA is integrated into wasp genomic DNA and excised and circularized only in females (A. Gruber, this meeting). At oviposition PDV are injected along with venom and the parasitoid egg into the host egg. On the basis of Southern blot analyses we show that viral DNA persists in the course of host larval development in several tissues.

Furthermore data on transcription of viral DNA will be presented. PDV are indispensable for parasitoid development as they suppress the immune reaction of the host against the parasitoid larva. Comparative analyses of morphology, spreading behaviour and relative abundance of the various types of haemocytes revealed no major differences between non-parasitized and PDV/venom containing larvae. Also, encapsulation experiments indicated that PDV/venom do not generally suppress the immune system of the host but specifically prevent encapsulation of the parasitoid larva. Another function of PDV/venom in this egg-larval parasitoid is induction of a developmental arrest in the prepupal stage, a phenomena also observed in several larval parasitoids.

05-007

DEVELOPMENTAL AND PHYSIOLOGICAL EFFECTS ON HOSTS INDUCED BY CHELONINE WASPS

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Parasites in the subfamily Cheloninae cause several developmental and physiological changes in their hosts, and impart a number of regulatory agents to the host to cause these effects. These effects include precocious initiation of metamorphosis, suppression of prepupal development, host castration and suppression of the host immune response. These effects are seen both in hosts containing a live endoparasite larva ("truly parasitized") as well as in hosts containing no live endoparasite ("pseudoparasitized"). A number of laboratories have addressed the source of these effects in species of *Chelonus*, *Phanerotoma* and *Ascogaster*. While it is possible to cast the various results in a manner that creates the appearance of a particular contradiction, such impasses may be more apparent than real, and where possible it is more informative to discern a harmonious explanation that sheds light on what effects are actually happening. In one effect to be discussed, high ecdysteroid measurements on truly parasitized prepupae may appear not to support measurements of low ecdysteroid titers in pseudoparasitized prepupae. However, these results were seen to be harmonious when it was determined that what is actually happening is that the parasite larva secretes ecdysteroids into the hemolymph of the host, whose endogenous ecdysteroid titer has been suppressed. Application of this methodology to the experimental results reported from various chelonine-host systems productively generates an embrative model for the underlying biochemical and physiological interactions.

05-008

SELECTIVE ACTION OF TERATOCYTE SECRETORY PRODUCTS ON HOST AND NON-HOST TISSUES.

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In the host parasitoid association *Heliothis virescens* (F.) - *Microplitis croceipes* (Cresson), teratocytes play a role that is apparently independent of the parasitoid larva and accompanying polydnavirus and venom injected into the host at the time of oviposition. The most obvious effects are inhibition of larval growth and pupation. The hemolymph from both parasitized larvae and larvae injected with physiological doses of teratocytes have depressed levels of juvenile hormone esterase (JHE) and arylphorin, although their respective mRNAs are reduced only in parasitized larvae. The reduction of arylphorin mRNA level is tissue-specific because no reduction was observed in testes from the same parasitized larvae. Our data suggests that teratocytes may negatively regulate a post-transcriptional mechanism. Teratocyte secretory products (TSP), obtained from *in vitro* cultures of teratocytes, inhibit the *in vitro* production of JHE by fat body in a dose dependent manner. In addition, TSP inhibits the incorporation of [³⁵S]-methionine into proteins when used in *in vitro* tissue assays of both fat body and larval testes. With respect to transcriptional and post-transcriptional events, we know that the responses of parasitized larvae and larvae injected with teratocytes are different. We examined the effects of TSP on the ability of fat body, testes, salivary gland, midgut and epidermal tissues from *H. virescens* and *Helicoverpa zea* (Boddie) (permissive hosts for *M. croceipes*) and *Manduca sexta* (L.) (nonpermissive host) to synthesize protein. Similar tissues from parasitized and nonparasitized *H. virescens* larvae were compared. Some tissue types from permissive hosts were more sensitive to TSP while the nonpermissive host tissues showed little response. Tissue response also depended upon the developmental age of the tissue. Fat body and testes were the most sensitive tissues. TSP effects on JHE synthesis by fat body of the three species was also measured.

05-010

PROTHORACIC GLAND INACTIVATION IN *HELIOTHIS VIRESCENS* (F.) (LEPIDOPTERA, NOCTUIDAE) LARVAE PARASITIZED BY *CARDIOCHILES NIGRICEPS* VIERECK (HYMENOPTERA, BRACONIDAE)F. Pennacchio, P. Falabella, R. Sordetti, C. Malva¹, S. B. Vinson²
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Heliothis virescens (F.) larvae parasitized by the endophagous braconid *Cardiochiles nigriceps* Viereck become developmentally arrested as last instars and fail to pupate. The biosynthetic activity of prothoracic glands of mature larvae parasitized, or treated with *C. nigriceps* calyx fluid and venom, is severely depressed, even though their histological degeneration is not observed and only minor ultrastructural changes are evident. This functional block is due to an alteration of the signal transduction pathway under PTTH control. The increased endocellular level of cAMP, induced by PTTH stimulation, fails to activate the cAMP dependent protein kinase, resulting in a marked underphosphorylation of target proteins. This is associated to a translational block of protein synthesis, which is a fundamental step of steroidogenic responses in animals. Time-course studies demonstrated that this alteration can be observed within 12 hr from parasitoid oviposition into host last instar larvae. Southern blot experiments indicate the presence of a PKI (protein kinase inhibitor)-like gene in *C. nigriceps*. PKIs so far isolated and characterized have a pseudosubstrate structural domain, which could inactivate a protein kinase by folding back into the active site. Further studies are in progress to characterize this parasitoid gene and to assess if it could be involved in prothoracic gland inactivation of parasitized *H. virescens* last instar larvae.

05-009

REGULATION OF HOST (*MANDUCA SEXTA*) HEMOLYMPH ECDYSTEROID LEVELS BY THE PARASITIC WASP, *COTESIA CONGREGATA* (LEPIDOPTERA: SPHINGIDAE - HYMENOPTERA: BRACONIDAE)D. B. Gelman, T. J. Kelly, D. A. Reed¹, N. E. Beckage¹INHL, ARS, USDA, Beltsville, MD, USA, and ¹Department of Entomology, University of California, Riverside, CA, USA

When parasitized, last-instar *Manduca sexta* larvae undergo developmental arrest. Neither a premolt hemolymph ecdysteroid peak nor a pupal molt is observed. A small peak of ecdysteroid is present in host hemolymph 12 to 24 hours prior to parasite emergence and the parasite's concurrent molt to the third instar. Ecdysteroids present in this host peak were 20-hydroxyecdysone, 20,26-dihydroxyecdysone, one or more very polar ecdysteroids, and small quantities of 26-hydroxyecdysone and ecdysone. Ligation experiments suggested that the parasite is partially responsible for the observed ecdysteroid peak. To clarify the mechanism by which the parasite controls ecdysteroid levels in the host, a larval *in vitro* prothoracic gland assay was developed to compare levels of prothoracicotropic hormone in brain extracts prepared from parasitized and unparasitized 5th instars. For unparasitized larvae (wandering +2 days) and host larvae (7 days = emergence of parasites +3 days), activity per brain was similar. However, there was a significant difference in the ability of prothoracic glands (cultured *in vitro*) from young 5th instar parasitized and unparasitized larvae to respond to brain extract; those from parasitized larvae exhibited little response. (Supported in part by NRI 92-37302-7470 to N. B.)

05-011

MECHANISM OF PARASITISM-INDUCED ELEVATION OF HAEMOLYMPH GROWTH-BLOCKING PEPTIDE LEVELS IN HOST INSECT LARVAE.

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Parasitism of *Pseudaletia separata* larvae by the parasitoid wasp *Cotesia kariyai* causes a delay in pupation of the host last instar larvae. Previous studies revealed that parasitism elevates the level of a biogenic peptide, growth-blocking peptide (GBP), in haemolymph of the host larvae which disturbs the normal development of the last instar larvae through repression of plasma JH esterase activity, and consequently delays in pupation. Recently we demonstrated that GBP mRNA is constantly expressed in both parasitized and nonparasitized last instar larvae and there is no difference in the levels of the mRNA between both larvae, thus indicating that parasitism may not effect on transcriptional level to elevate haemolymph GBP concentration. The present study was conducted to reveal the detailed mechanism by which parasitism elevates haemolymph GBP level.

05-012

PARASITISM-INDUCED HOST PHYSIOLOGICAL SABOTAGE IN *MANDUCA SEXTA*: AN INTIMATE LOOK AT HOW PARASITOIDS KILL HOSTS

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The braconid wasp parasitoid *Cotesia congregata* and its associated polydnavirus impact the host's physiology via multiple mechanisms. The PDV has an immediate 'knock-out' effect on the host's immune system, causing apoptosis of the hemocytes which would otherwise avidly encapsulate the wasp eggs. The PDV-encoded 'early protein 1' is linked to successful development of the parasitoids; recent evidence suggests that EPI-like sequences are also present in other parasitoids. Later, the parasitoids evade encapsulation via non-virally mediated mechanisms. The PDV also induces host developmental arrest, which is elicited by neuroendocrine and endocrine disruption, but the virus does not appear necessary to trigger cessation of host food consumption preparatory to wasp emergence. Surrogate hosts implanted with parasitoids also cease feeding prior to their emergence. The host's respiratory physiology is altered in that the pattern of ventilation becomes transiently discontinuous as the wasps are emerging from their host, and at other times the rate of carbon dioxide emission is extremely low, providing evidence for a low metabolic rate during the final stages of parasitism. In its last instar, the host's mass is directly proportional to the number of parasites developing within it, arguing that there is a wasp 'dose-dependent' effect on host growth. The presence of the parasitoids is required for induction of testicular atrophy, which occurs in the host's final instar prior to emergence of the wasps; instead of proliferating as normal, the testes diminish in volume. Both the virus and the parasitoids cause changes culminating in host death.

05-014

REGULATION OF TESTIS GROWTH OF THE HOST, *PSEUDALETIA SEPARATA* BY THE POLYDNAVIRUS AND VENOM OF *COTESIA KARIYAI*.

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The larval endoparasitoid must struggle for the resource against the host. If a part of host resource is deprived of the host by itself, its resource can not be available for the parasitoid. The male lepidopteran host develops the testis during the larval stage. If a parasitoid gives the host development leave, the parasitoid loses a part of the resource. The parasitoid, *Cotesia kariyai* suppressed the development of the host testis through polydnavirus plus venom. Its degree of suppression was dose-dependent response of the polydnavirus plus venom. The expression of polydnavirus mRNA had a peak in 12 hrs after parasitization. It was shown by the paraffin sections and the cell sorting results that the polydnavirus may arrest the meiosis and/or mitosis on the cell cycle during the developing testis of the host.

05-013

ABERRANT NUTRITIONAL REGULATION OF *DE NOVO* CARBOHYDRATE SYNTHESIS IN PARASITIZED *MANDUCA SEXTA* L.

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De novo glucose/trehalose synthesis via gluconeogenesis from amino acids by *Manduca sexta* is dependent on nutritional status. Gluconeogenesis from (3-¹³C)alanine is only induced in normal 5th instar larvae in the absence of dietary carbohydrate, while glucogenesis in insects maintained on a high carbohydrate diet can be accounted for by metabolism of alanine through the tricarboxylic acid cycle. The present studies demonstrated that this nutritional regulation is upset in *M. sexta* parasitized by *Cotesia congregata*. In parasitized larvae significant gluconeogenesis from labeled (3-¹³C)alanine and (2-¹³C)pyruvate was evident in larvae maintained on either a high or low carbohydrate diet. The TCA cycle contribution to glucogenesis in parasitized insects on the high carbohydrate diet was approximately 50%. On the low carbohydrate diet the contribution of the TCA cycle to trehalose labeling was negligible. Pentose cycling was observed in larvae on either diet, but parasitism had no significant effect, even on the high carbohydrate diet. Based on the asymmetric ¹³C distribution in trehalose the cycling rate was approximately 50% in larvae on the high carbohydrate diet and 10% in larvae on the low carbohydrate diet. The possible bases for this metabolic alteration and its potential significance are discussed.

05-015

THE PHYSIOLOGICAL CHARACTERISTICS OF THE VENOM OF THE EULOPHID PARASITOID *EUPLECTRUS COMSTOCKII*: A CHRONICLE OF VENOM PROTEINS, VENOM ACTIVITY AND STORAGE PROTEIN TITER WITHIN THE HEMOLYMPH OF *TRICHOPLUSIA NI*.

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The ectoparasitoid *Euplectrus comstockii* (Hymenoptera: Eulophidae) injects a venom into the hemocoel of its hosts where the hemolymph is believed to facilitate the transport of the venom to target tissues. The wasp's venom alters the physiology of the host to accommodate its young by arresting larval-larval ecdysis and stimulating the premature production of storage proteins. To further determine whether these effects are due to one or more substances within the venom, the temporal dynamics of venom activity within the hemolymph of the host was investigated by bioassaying parasitized hemolymph at different times post-parasitism for arrestment activity, and for determining storage protein production. Crude venom, extracted from *Euplectrus* venom glands, was also incubated in hemolymph or phosphate buffer outside the host, and bioassayed as well. These studies show that arrestment activity within the hemolymph decreases to < 5% within 12 hours post parasitism, while purified venom incubated in a phosphate buffer solution retains 100% of its arrestment activity over this time period. A profile of venom proteins present in parasitized hemolymph which arrests molting or stimulates storage protein production was compared to the venom profile from inactive hemolymph. These results provide further clues as to the function of specific proteins within the venom of *Euplectrus comstockii*.

05-016

HOST CASTRATION, DIAPAUSE, AND ECDYSTEROIDS; NATURAL SELECTION REFINES THE HOST/PARASITOID INTERACTION.

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My narrative explores a possible sequence of intermediate rewards to the castrator. These include:

1) the diversion of energy reserves away from host reproductive tissue for the benefit of the parasitoid larva. 2) The elimination of host testes that are involved with ecdysteroid metabolism, which could impair a parasitoid larva's ability to monitor the host's endocrine system in order to synchronize its own overwintering survival and vernal development with that of its host. 3) Host testes may interfere with the parasitoid's pre-egression control of apolysis of the host's integument, thereby impeding the ability of the newly molted third stadium parasitoid to egress from the host's hemocoel. These intermediate steps may have culminated in the ultimate reward for the species, *i.e.* the deterrence of host resistance to the parasitoid. The ability to castrate hosts is the result of group selection, but is probably the end product of multiple steps. As a parasitoid species masters each of the three individual evolutionary strategies mentioned above, it would have more offspring than a competitor species which has not mastered its niche.

05-018

A POSSIBLE MECHANISM FOR THE PHYSIOLOGICAL SUPPRESSION OF CONSPECIFIC AND HETEROSPECIFIC EGGS FOLLOWING SUPERPARASITISM OR MULTIPARASITISM BY COMPETING ENDOPARASITIDS

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The progeny of endo-parasitoids that oviposit in already parasitized hosts often fail to hatch. This occurs regardless of whether the host was parasitized sometime earlier by a conspecific or heterospecific female. Although in many cases one of the parasitoids hatch and physically attack and kill the embryo in the egg before it can hatch, in other cases there is no evidence of any physical damage. In these later cases the death of the egg has been attributed to the release of toxins by the older developing parasitoid, anoxia due to the presumed more efficient absorption of oxygen by the older parasitoid, or the absorption of essential nutrients by the older parasitoid required by the developing egg. We provide some evidence for an alternative hypothesis concerning the physiological suppression of younger eggs developing in super- or multi-parasitized hosts.

05-017

PHYSIOLOGICAL INTERACTIONS BETWEEN *GLYPTAPANTELES LIPARIDIS* (HYMENOPTERA: BRACONIDAE) AND ITS HOST LARVA, *LYMANTRIA DISPAR* (LEPIDOPTERA: LYMANTRIIDAE)

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The influence of parasitism by the gregarious braconid, *Glyptapanteles liparidis*, on development, nutrition, and the juvenile hormone (JH) titer of its host larva, *Lymantria dispar*, was studied in two different host types, which were characterized by the stage of parasitization: A-hosts were parasitized during premolt into the 2nd instar, B-hosts during premolt into the 3rd instar. In the normal pattern the developmental of parasitoid larvae is extended over three host instars. The relatively constant time required by *G. liparidis* for its endoparasitic development causes varying nutritional conditions for the host-parasitoid complex, depending on the relationship between the host's size and the volume of the parasitoid complex inside the host. Thus, only early parasitized hosts showed increased rates of consumption and growth during the 3rd, penulti-mate host instar and a prolonged development during their final 4th instar. The approximate digestibility (AD-value) and conversion efficiency of ingested and digested food (ECI- and ECD-values) was uninfluenced by parasitism.

The hemolymph trehalose level the host larva was markedly reduced, when the parasitoid larvae had reached their second instar. Compared to unparasitized larvae, a markedly lower glycogen content was only found in newly molted third instar A-hosts. Final fifth midinstar B-hosts even showed a significantly higher mean glycogen level than unparasitized larvae.

Simultaneously to the reduction of the trehalose level, the JH titer increased in host larvae, when the parasitoid larvae had molt into the 2nd instar. This was especially obvious in A-hosts during the late endoparasitic period, when the JH III titer rose to values of 200-500 pmol/ml while it remained below 1 pmol/ml in unparasitized larvae. Parallel to this increase a smaller one could also be observed in the JH II titer, which is the predominant JH homologue in unparasitized gypsy moth larvae. Compared to unparasitized larvae, a generally reduced activity of JH esterase (JHE) is found in parasitized larvae throughout the pen-ultimate and final larval stages, which may be in part responsible for the increased JH II and JH III titers.

05-019

PROTON POWER AND ANIMAL PLASMA MEMBRANES

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Proton-motive forces energize animal plasma membranes throughout the animal kingdom. Six examples will be discussed - insect midgut alkalization, vertebrate kidney acidification, mammalian epididymal acidification, mammalian phagocytosis, mammalian bone resorption, and frog skin Na⁺ uptake. The electrogenic H⁺ V-ATPase consists of several subunits arranged in a peripheral V₁ domain, including a stalk that connects it with a membrane bound V₀ domain. In midgut the H⁺ V-ATPase energizes amino acid uptake and, in cooperation with an electrophoretic K⁺/H⁺ antiporter, alkalizes the lumen. In kidney ATPases are recruited to the apical membrane during acidosis and several mechanisms for regulating their activity are known. In epididymis, the enzyme produces an acid environment that is responsible for male fertility. In phagocytes the enzyme energizes microbicidal and tumoricidal activity. In bone the enzyme secretes acid which resorbs Ca²⁺ and which activates enzymes that break down the organic matrix. In frog skin the enzyme energizes Na⁺ uptake from ponds. In the same sense that energization of basolateral membranes is widely held to use Na⁺/K⁺ P-ATPases, energization of many apical membranes appears to use H⁺ V-ATPases.

05-020

CHARACTERIZATION AND LOCALIZATION OF V-ATPASE SUBUNITS IN THE TOBACCO BUDWORM AND IN MOSQUITOES

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The transmembrane sector of V-ATPases is involved in proton conduction across the membrane where a 15-17 kDa proteolipid forms a putative proton channel. We previously reported the cloning of a 17kDa protein from *Heliothis virescens* which is highly homologous to the published sequences of other 17kDa proteins. An affinity-purified rabbit polyclonal antibody was developed to an antigenic and putatively extracellular region of a cloned 17 kDa proteolipid. In larval tissue sections this antibody labeled the midgut goblet cell apical membrane in *H. virescens* and the apical membrane in Malpighian tubules from *H. virescens* and *Manduca sexta*. The antibody also recognized the 17 kDa protein in an immunoblot of *H. virescens* Malpighian tubule homogenate. Immunolocalization of this protein in the midgut during the L₄-L₅ larval molt and early post ecdysis into the fifth instar in *H. virescens* indicates that the spatial expression of the 16kDa protein is developmentally regulated. Northern blot analysis revealed the presence of two transcript sizes in the midgut and Malpighian tubules. Our results strongly support the hypothesis that the 17 kDa protein is a component of the V-ATPase, where it is thought to be the proton-conducting subunit.

V-type ATPase subunits have also been cloned from the Malpighian tubules of mosquito larvae. Two of the subunits cloned show high homology to the subunits previously cloned from other lepidopteran insects.

05-022

MOLECULAR PHYSIOLOGY OF V-ATPASE FUNCTIONINGS IN THE SILK GLAND CELL OF *BOMBYX MORI*

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An active transport system of vacuolar-type H⁺-ATPase (V-ATPase) was identified and characterized in the anterior silk gland of 5th instar silkworm larva, *Bombyx mori*. Using fluorescent acridine orange, acidic granules were found in the apical region of cytoplasm. The acidification of these granules was completely inhibited under the presence of 0.1-1 μ M bafilomycin A1, reversibly. This was observed throughout the larval stage of 5th instar until the onset of spinning. During the incubation with acridine orange, acidic granules seemed to be transported apically and to be concentrated beneath the apical plasma membrane. This implies that V-ATPase is shuttled between an intracellular vesicular compartment and the plasma membrane. The microsomal membrane vesicles from the anterior silk gland cell showed ATP-dependent H⁺-transport activity, which was greatly enhanced by the presence of Cl⁻. The immunological blotting test with the antibody against V-ATPase holoenzyme of *Manduca sexta* midgut showed the presence of subunit A, B, C and G of V-ATPase in the anterior silk gland cell. Molecular cloning has been done on the subunit G (13-kDa). The coding region of the cDNA showed almost identical amino acid sequences with *Manduca* midgut subunit G. Molecular genetic studies are in progress using this clone. The cellular function of V-ATPase in this tissue will be discussed, but several lines of evidences suggest that the V-ATPase of the anterior silk gland might participate in the acidification of the glandular lumen during the feeding period of 5th instar.

05-021

POSTTRANSCRIPTIONAL REGULATION OF THE INSECT V-ATPASE

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The plasma membrane V-ATPase in the midgut of *Manduca sexta* is a multisubunit enzyme comprised of a peripheral, catalytic V₁ and a membrane bound, proton translocating V₀ complex. It is located in the apical membranes of goblet cells, where it energizes K⁺ secretion by driving electrophoretic K⁺/2H⁺ antiport. During moult, V-ATPase activity is regulated by dissociation of V₁ subunits from the V₀ complex (Sumner et. al. (1994) J. Biol. Chem. 270, 5649-5653).

We have purified and characterized a soluble V₁ complex from the cytosol of midgut cells. It is a major cytosolic protein and exhibits Ca²⁺, but no Mg²⁺ dependent ATPase activity. 25 % methanol restored Mg²⁺ dependent ATPase activity. The cytosolic complex consisted of five polypeptides, the subunits A, B, E, F and the novel 13-kDa subunit G. During moult, the amount of cytosolic V₁ complex increased twofold as compared to the amount found in intermoult larvae. Starvation of intermoult larvae led to the same amount of the cytosolic complex as during the moult, indicating similar regulatory mechanisms.

In contrast to the peripheral V₁ complex only little is known about the membrane bound V₀ domain. In a first attempt we have focussed on the membrane associated 39-kDa subunit a. Screening of a midgut cDNA library revealed several clones, two of which were partially sequenced. One clone represented the cDNA encoding subunit a, with a derived amino acid sequence exhibiting 81% identity to the corresponding bovine and human cDNAs. The second clone represented a polyadenylated antisense transcript for subunit a. Northern blots showed three transcripts for the sense (1.5, 1.8 and 2.2 kb), but none for the antisense strand. However, the presence of antisense mRNA could be detected by RT-PCR. This result might reflect antisense regulation for subunit a expression. Since this subunit may contribute to the link between V₁ and V₀ domain, its posttranscriptional regulation could also influence the assembly of the holoenzyme.

05-023

A GENETIC MAP OF THE *DROSOPHILA MELANOGASTER* MALPIGHIAN TUBULE

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Physiology tends to be analytical; that is, the problem is simplified in order to make it tractable. Frequently, this leads to a progression from whole animal to cell culture, either of cells from the tissue in question and expressing at least some differentiated property of interest; or of a cloned gene expressed heterologously in some bland background (e.g. *Xenopus* oocytes) that lack complicating extraneous factors. This analytical approach, however, does not lend itself to study of complex tissues and their control, precisely because of the nature of living systems. In order to study these problems in integrative physiology, we must be able to manipulate gene expression in specified tissues in the living organism.

Accordingly, we have spent the last four years developing an epithelial model which is uniquely amenable to physiological, biochemical, molecular and genetic analysis. The *Drosophila melanogaster* Malpighian tubule is the smallest insect epithelium yet studied; it is a simple epithelium of 145±1 principal cells. However, it is able to secrete fluid faster (on a per-cell basis) than any other known epithelium. Fluid secretion is driven by a plasma membrane V-ATPase, and is controlled by the cAMP, cGMP/nitric oxide and intracellular calcium signalling systems. Using enhancer-trap techniques, we have demonstrated previously unsuspected heterogeneity in this apparently simple epithelium, and we have been able to map boundaries to single cell resolution. The same technology now allows us to express genetic constructs of our choice in any tubule region or cell type that we have identified, and so provides a framework for novel experiments in integrative physiology that is not available for other tissues or other organisms.

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05-024

DOES AMINOPEPTIDASE N PLAY A ROLE IN AMINO ACID TRANSPORT ALONG THE MIDGUT OF *BOMBYX MORI* LARVAE?

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The aminopeptidase N catalyses the removal of amino acids from peptide substrates and has a broad specificity, preferring peptides with neutral N-terminal amino acids. Recently, it has been proposed that aminopeptidase N could be functionally linked to amino acid transport system in mammalian epithelia [Plakidou-Dymock et al., Biochem. J. 290 (1993): 59-65].

In lepidopteran midgut amino acid absorption occurs through a K⁺-dependent symport and aminopeptidase N represents one of the most abundant membrane enzyme. This peptidase was not removed by treatment with papain as in mammalian tissues, but it is partially released (up to 40% in *Bombyx mori*) after incubation with phosphatidylinositol-dependent phospholipase C. After the partial release of aminopeptidase N amino acid transport activity was unchanged. Therefore to explore the presence of a functional link between the aminopeptidase N and the lepidopteran K⁺/amino acid symport, kinetics experiments were performed for both of them. The affinities of neutral amino acids, amino acid analogues and inhibitors were assayed on both purified *B. mori* aminopeptidase N and the K⁺/amino acid symport. Our results failed to demonstrate any kinetic association between aminopeptidase and amino acid transport activities. Major evidences can be summarised as follows: i) bestatin and Zn⁺⁺ are strong inhibitor of aminopeptidase N activity at concentrations not affecting amino acid symport; ii) bestatin binds to and inhibits the K⁺/amino acid symport with a higher K_i than that for aminopeptidase N; iii) several leucine analogues are valid substrates only for the symporter; iv) the K⁺/amino acid symport appears strongly dependent on membrane electrical potential and an alkaline external pH.

05-025

AMINO ACID TRANSPORT IN COLEOPTERAN MIDGUT

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Fundamental differences in midgut structure, physiology, brush border proteins and transporters among the coleopteran, *Leptinotarsa decemlineata* and other insect taxa are reviewed. *L. decemlineata* utilizes both ion-dependent and ion-independent transport systems. Like lepidopteran midgut ion-dependent transporters are stimulated by both Na⁺ and K⁺. K⁺-stimulated amino acid uptake into brush border membrane vesicles is the same in the presence or absence of a gradient but is slowed by an outward cation gradient. Because amino acids do not concentrate in *L. decemlineata* BBMVs, the *Bacillus thuringiensis* CryIIIA toxin does not inhibit ion-dependent uptake of amino acids. A model of amino acid transport in *L. decemlineata* is discussed.

05-026

GLUTAMATE TRANSPORT AND STORAGE BY VARIOUS INSECT EPITHELIA

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Notably absent from the amino acid-rich blood plasma of caterpillars are significant amounts of two neuroactive dicarboxylic amino acids, L-aspartate and L-glutamate. Several tissues of the *Calpodex ethlius* caterpillar, such as the epidermis and the epithelia associated with the rectal complex, form large fluid-filled extracellular compartments that are isolated from the blood plasma and that periodically sequester millimolar concentrations of these two amino acids, presumably by transport mechanisms present in the epithelial plasma membranes. In the prepupal epidermis, glutamate storage in the ecdysial space peaks about 6 hr before pupation, but disappears rapidly and just before pupal ecdysis. The kinetics of glutamate reabsorption by the epidermis differs from that of other amino acids present in the ecdysial fluid, and uptake is independent of water removal from the ecdysial space by the caterpillar. In the subcuticular compartment of the rectal complex of the feeding caterpillar the concentration of these two amino acids climbs to 300-fold the micromolar levels found in the blood plasma and then drops to plasma values. This cycle of glutamate storage appears to be synchronized to the rectal cycle of water uptake associated with defecation. Alkaloids present in the food plant may also accumulate in the rectal complex during this cycle. The nature of the epithelial glutamate transport mechanisms involved will be discussed and contrasted with those of other insect tissues. These compartments may serve to provide large amounts of readily accessible glutamate for energy metabolism in these epithelia. The periodic nature of this storage suggests that epithelial activity is controlled by the cyclical release of neuropeptides that trigger glutamate transport into and out of these subcuticular spaces.

05-027

LOCUST ION TRANSPORT PEPTIDE: FUNCTION, PRIMARY STRUCTURE, CLONED DEOXYRIBONUCLEIC ACID AND EXPRESSION.

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Locust Ion Transport Peptide (ITP) purified from corpus cardiacum (CC) of *Schistocerca gregaria* stimulates Cl⁻, Na⁺, K⁺ and fluid reabsorption and inhibits acid secretion in anterior hindgut, which is functionally equivalent to the proximal tubules of vertebrate nephrons. A partial amino acid sequence and the polymerase chain reaction were used to obtain ITP cloned deoxyribonucleic acid (cDNA) encoding a 130 amino acid (aa) prohormone from which ITP is cleaved. ITP is a neuropeptide of 72 amino acids with terminal amidation, 3 sulphur bridges and a reduced molecular weight of 8564 Daltons. Insect Sf9 cells transfected with ITP cDNA using a baculovirus secreted a biologically active stimulant of locust hindgut transport. ITP synthesized by David King behaves like our purified ITP. ITP mRNA was detected in only brain and CC tissues. An ITP-related mRNA is present in several non-nervous tissues that do not stimulate the hindgut bioassay and its protein product (ITP-L) may have a negative feedback function. The ITP-L prohormone is 4 aa's longer than ITP and only 15 of the C-terminal 40 aa's are conserved relative to ITP. ITP has high sequence homology with a family of crustacean hormones having hyperglycaemic (CHH), molt-inhibiting (MIH) and vitellogenesis-inhibiting (VIH) actions (Meredith et al., J. Exp. Biol., In Press). MIH and CHH do not stimulate locust ileal Cl⁻ transport, but do inhibit such stimulation by ITP. A putative antidiuretic neuropeptide from *Locusta migratoria*, neuropepsins, does not stimulate either Cl⁻ or fluid absorption in hindgut of *Schistocerca gregaria*.

05-028

FIRST PRINCIPLES IN EPITHELIAL TRANSPORT PHYSIOLOGY: ELECTRICAL COUPLING OF TRANSCELLULAR AND PARACELLULAR TRANSPORT PATHWAYS

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Electroneutrality of solutions requires that for every cation transported across an epithelium an anion is moved in the same direction or another cation is moved in the opposite direction. In the case of Malpighian tubules, the secretion of K^+ and Na^+ into the tubule lumen is accompanied by an equivalent secretion of Cl^- or other ions. Since transepithelial secretion of K^+ and Na^+ is active (against electrochemical potentials), secretion of these cations must be transcellular, i.e. through epithelial cells that deliver the energy required for transport. In contrast, transepithelial secretion of Cl^- is passive (down the electrochemical potential). Requiring no cellular energy, transport of Cl^- may be paracellular, i.e. between epithelial cells (through septate junctions). Thus, active and passive transport pathways are parallel to each other. They form an intraepithelial electrical circuit where the transcellular active pathway may be described to consist of an electromotive force (E) and a transcellular resistance (R_c), while the paracellular passive pathway consists of only a shunt resistance (R_{sh}). Current in the circuit is equivalent to rates of transepithelial cation and anion secretion and regulated by changing E (metabolism, pumps), R_c and R_{sh} (conductances, channels). Mosquito natriuretic peptide affects primarily R_c , while leucokinin affects primarily R_{sh} , and CCRF-like diuretic peptide affects both in a dose dependent fashion. Effects on E, R_c and R_{sh} are additive, endowing the epithelium with transport rates that range from antidiuresis to diuresis.

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05-030

ACID-BASE RELATIONS BETWEEN GUT, MALPIGHIAN TUBULES AND HAEMOLYMPH IN LEPIDOPTERAN LARVAE: SOME PIECES OF THE PUZZLE.

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The pH of body fluid compartments of 5th instar larvae of the tobacco hornworm *Manduca sexta* (Lepidoptera: Sphingidae) was evaluated by dissection after injection of pH-sensitive phenol red dye into the haemolymph. Consistent with previous work, the midgut contents are strongly alkaline and the ileal and rectal contents strongly acid throughout the 5th instar. In 5th instar larvae weighing <3 gm, all segments of the Malpighian tubules contain weakly acidic fluid; the midgut contents are strongly alkaline and the rectal contents are strongly acidic. At 3-4 gm body weight the ileal plexus fluid and the ileal tissue itself become strongly alkaline, indicating an increase in alkali recovery from the ileal contents. This picture is consistent with recycling of alkali from hindgut to midgut for resecretion, starting in the latter part of the 5th instar.

The midgut's alkali secretion rate *in vivo* was conservatively estimated at 54 μ Eq/hr for 8-10 gm animals, whereas the rate for isolated midgut is at least 10fold lower, even in solutions containing protein hydrolysate or haemolymph. The latter rate was insensitive to imposed transepithelial potentials, ruling out a passive contribution. To identify possible stimulatory or cooperative factors involved in alkali secretion *in vivo*, two *in situ* preparations were used; "headless", in which the midgut was cannulated from the anterior end of the animal, and "tailless", in which the last three segments were removed and the midgut was cannulated from the posterior end. Tailless preparations alkalinized injected solution markedly better than headless preparations, suggesting that head structures (e.g. Brain, salivary glands) are important for alkalinization, whereas recycling of alkali from the hindgut is not essential, at least in the 10 to 30 minute time scale of these experiments.

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05-029

HOW IS FLUID SECRETION BY INSECT MALPIGHIAN TUBULES CONTROLLED?

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The Malpighian tubules of *Drosophila* are controlled by the cyclic AMP, cyclic GMP and calcium pathways. Using microelectrode techniques, we investigated whether they acted specifically on either cation or anion transport, or whether they activated both systems.

Stimulation with cyclic nucleotides alters the potential across principal cells, through the selective activation of an apical electrogenic V-ATPase. By contrast, manipulation of extracellular chloride levels, combined with stimulation with leucokinin, does not affect the potential profile across the principal cells, showing that chloride must pass through another route.

The cell-permeant calcium chelator, BAPTA-AM, was shown to suppress the action of leucokinins but not those of cAMP, or the neuronally-derived insect peptide CAP_{2b} or its intracellular messenger cGMP. This shows that leucokinins act through calcium and not through cyclic nucleotides, and that the cyclic nucleotide pathways do not co-activate the intracellular calcium pathway to exert their effects.

Taken together, these results show that leucokinin acts through intracellular calcium, independently of cAMP or cGMP, to raise chloride permeability of the epithelium. By contrast cyclic nucleotides act on the electrogenic cation transporting apical V-ATPase, with only a negligible effect on anion conductance, and without perturbing intracellular Ca^{2+} .

References:

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05-031

AMMONIA IS A FACTOR IN ALKALINIZATION OF MIDGUT CONTENTS BY *MANDUCA SEXTA* LARVAE (LEPIDOPTERA:SPHINGIDAE)

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Lepidopteran larvae sustain luminal pH values as high as 10-11 in the anterior and middle midgut and values of 8-9 in the posterior midgut. These values are among the highest in living animals and cannot be explained by the transport processes presently known from studies of the midgut epithelium *in vitro*. We observed that the pH of fresh midgut contents from 5th instars of the tobacco hornworm undergoes a rapid drop of 2-3 pH units upon degassing, indicating loss of volatile alkali. Fresh midgut contents were collected directly into 0.1 N nitric acid and total ammonia content was determined from the formation of indophenol. Total ammonia ($NH_3 + NH_4^+$) averaged 2.63 ± 0.135 mM in anterior, 2.88 ± 0.304 in middle, and 1.92 ± 1.74 in posterior midguts. The partial pressure of NH_3 was calculated on the assumption that the pH was 10 in anterior and middle and 8 in posterior midguts. In anterior midgut, P_{NH_3} averaged 86.9 ± 0.97 mm Hg or more than a tenth of an atmosphere. Average P_{NH_3} in middle midgut was 80.6 ± 16.0 mm Hg. Because of the lower pH in posterior midgut, the average P_{NH_3} was 3.8 mm Hg. The high values are nonetheless underestimates, since some constituent of the gut contents destroys the indophenol produced in the assay. We propose that *in vivo* alkalinization includes two processes. One involves secretion of fixed alkali and may occur by mechanisms suggested by previous workers. This process takes dietary material from its initial pH value of 5.25 to approximately 8.0. The second process involves either direct secretion of NH_3 by the midgut epithelium or enzymatic production of NH_3 within the gut contents. This process brings the pH of gut contents to above 10.

05-032

ONTOLOGY OF RECTAL WATER VAPOUR ABSORPTION

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The eggs and the short-lived first instar nymph of the firebrat, *Thermobia domestica*, are incapable of absorbing water vapour. They owe their survival in desiccating conditions to the water reserves supplied by the mother in the egg and to mechanisms for limiting transpiratory water loss. This is probably one reason for the very short-lived first instar. At this stage, only the rudiments of the proctodaeum have developed. Continuous mass records show low rates of mass (water) loss over long periods in high relative humidity (84% RH). Hatching and moulting are recorded as small abrupt mass losses, as hatching or moulting fluid evaporates, so readily distinguishing each instar.

During the second instar, onset of vapour absorption is shown by small sporadic bouts of mass gain, especially towards the latter part of the instar. This coincides with partial development of the posterior rectal sacs, which continue to develop throughout this instar and reach full development in the third and fourth instars. Third instar nymphs are capable of sustained vapour absorption over extended periods at high rates. Early instar nymphs reach maximal rates of absorption normalised to body mass well in excess of the maximal rates found in later nymphs and adults, when a linear allometric relationship is found. Maximal rates of absorption recorded for late instar nymphs and adult firebrats consistently approximate to 1% body mass per hour. Early second instar nymphs underperform against this standard but by mid-instar they breach this standard and by the end of the instar rates are more than doubled. In the third and fourth instars, maximal rates reach close to 3% body mass per hour. This early period of non-allometric development of vapour absorption is likely to be linked to the need for rapid and sustained early growth.

05-034

THE METABOLISM OF SUCROSE AND AMINO ACIDS AND OSMOREGULATION IN APHIDS

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Avoidance of dehydration of the haemolymph due to a very high dietary osmotic pressure is a major problem for aphids. The adaptations include a high osmotic pressure in the haemolymph and polymerisation of dietary sugars in the gut to form oligosaccharides. The rate of ingestion of a 25% w/v sucrose diet was $12.4 \text{ nl mg}^{-1} \text{ h}^{-1}$ and the excretion rate was $5.3 \text{ nl mg}^{-1} \text{ h}^{-1}$. Of the ingested diet 69% of the sucrose and 94% of the amino acids were assimilated. While feeding on a 25% w/v sucrose diet, sucrose was respired at the rate of $1.32 \times 10^{-6} \text{ mmol mg}^{-1} \text{ h}^{-1}$, equivalent to $0.35 \mu\text{l O}_2 \text{ mg}^{-1} \text{ h}^{-1}$. Sucrose was oxidised in preference to amino acids. The haemolymph of *A. pisum*, feeding on artificial diet containing 25% w/v sucrose, was found to contain two main sugars: trehalose (255 mM) and fructose (129 mM). No sucrose was found in the haemolymph. The honeydew sugars (350 mM) of aphids fed the same diet were mainly oligosaccharides (220 mM), which accounted for 85% of the total ^{14}C in the sample. It was estimated that the polymerisation of sucrose was responsible for a reduction in concentration of approximately 34%. At low dietary sucrose concentrations (5 to 10%), the honeydew contained mainly mono and disaccharides. At dietary sucrose concentrations of 15% and greater, oligosaccharides were predominant. This is consistent with the idea that osmoregulation is by oligosaccharide synthesis. Analysis of stomach contents revealed that oligosaccharide synthesis occurs there, while tissue incubation showed that the gut is much more active in oligosaccharide synthesis than the eviscerated body tissues. The function of the filter chamber, found in some aphid species, is considered and it is suggested that this is a mechanism for reducing the osmotic pressure of the ingested diet.

05-033

DISCONTINUOUS GAS EXCHANGE AND THE CUTICULAR AND RESPIRATORY COMPONENTS OF WATER LOSS IN THE COCKROACH *DIPLOPTERA PUNCTATA* (ESCHSCHOLTZ) (DICTYOPTERA: BLABERIDAE)

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Respiration and water efflux patterns in the pseudoviviparous cockroach *Diploptera punctata* (Eschscholtz) were measured simultaneously in a flow-through system using dual-wavelength infrared absorbance. Only cockroaches in a 'resting' posture exhibited cyclic discontinuous gas exchange with a frequency of 4.1 mHz. There was no obvious flutter phase prior to the main burst emission of CO_2 , however some individuals emitted several small bursts of CO_2 prior to the main burst. Unlike other insects, there was no relationship between the frequency of gas exchange cycles and the volume of CO_2 released. Therefore, to accommodate increasing CO_2 loss, *D.*

punctata increase both burst and interburst \dot{V}_{CO_2} . Water loss was constant during the interburst intervals representing cuticular water loss. Water loss increased from interburst levels only during bursts of CO_2 emission. Respiratory water loss accounted for only 8.4% of all water lost during a respiratory burst, and <5% of total body water loss. Discontinuous gas exchange in this species affords relatively little protection from desiccation.

05-035

LOCALIZATION OF AMINO ACID ABSORPTION SYSTEMS IN THE LARVAL MIDGUT OF *MANDUCA SEXTA*

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The midgut of *Manduca sexta* larvae is composed of three morphologically distinct regions: anterior, middle and posterior. The folding of the epithelium as well as the structure of columnar and goblet cells varies among the regions. In the middle region columnar cell structure changes gradually from anterior type to posterior type. On the basis of cell structure it has been postulated that nutrient absorption may be confined to the posterior type columnar cells.

Larval *M. sexta* midgut has been shown to contain at least three distinct systems for absorption of amino acids from the lumen contents by amino acid- K^+ symport. Brush border membrane vesicles (BBMV) were prepared separately from anterior and posterior midgut regions and assayed for the membrane marker enzyme aminopeptidase as well as for the presence and properties of the three amino acid- K^+ symport systems. Although the specific aminopeptidase activity in the posterior homogenate was nearly twenty times that in the anterior homogenate, enrichment in aminopeptidase activity was similar for BBMV from both regions. Symport systems for absorption of neutral amino acids were found only in posterior midgut BBMV. Proline uptake by anterior BBMV increased linearly with proline concentration but the dependence of the rate of uptake of other neutral amino acids on substrate concentration was biphasic; indicating the presence of a uniporter. Cationic amino acid- K^+ symport was found in both anterior and posterior BBMV. Substrate affinities were consistent with the same system being present in both anterior and posterior BBMV but the specific activity of the system was three times greater in the anterior BBMV.

05-036

CRYIAa δ -ENDOTOXIN FROM *BACILLUS THURINGIENSIS* EFFECTS K^+ /AMINO ACID SYMPORT IN *BOMBYX MORI* LARVAL MIDGUT.

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The inhibition of K^+ -dependent amino acid transport into brush border membrane vesicles (BBMV) from the midgut of susceptible lepidopteran larvae provides a reliable test for the evaluation of *Bacillus thuringiensis* (Bt) δ -endotoxin activity and specificity. An activated preparation of CryIAa toxin produced a specific dose-dependent inhibition of K^+ -dependent leucine transport into midgut BBMV from *Bombyx mori*. The activity of the δ -endotoxin was observed also in the absence of K^+ , which suggests its direct interaction with the K^+ /amino acid symporter (P.Parenti et al. J Invertebrate Pathol. 65,35-42, 1995). K^+ permeability and cation-dependent amino acid translocation in BBMV can be evaluated by monitoring the fluorescence of the voltage-sensitive cyanine dye 3,3'-diethylthiacarbocyanine iodide(DisC₃5). The symporter ability to accept Na^+ instead of K^+ was exploited and the dissipation of an imposed inside-negative potential (K^+ gradient in>out and valinomycin) was registered in the presence of a Na^+ gradient (out>in) and of the amino acid. The fluorescence quenching dissipated more rapidly when the amino acid was present. Preincubation of BBMV with CryIAa caused a significant decrease of the amino acid-dependent recovery of fluorescence, whereas K^+ permeability was unaffected. In the isolated midgut Bt δ -endotoxins inhibit leucine transepithelial fluxes as well as the electrical potential difference (PD). However the strong inhibition of amino acid flux showed a different timing compared to the drop of PD.

05-038

PLUTELLA XYLOSTELLA: MIDGUT ELECTROPHYSIOLOGY: TRANSEPITHELIAL & CELL RESISTANCES, EFFECTS OF K^+ & Ba^{++}

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Plutella xylostella (Px) midgut has a high transepithelial potential, V_{te} , of 40 mV (lumen positive) and resistance, R_{te} , of 174 $\Omega \cdot cm^2$ (De Decker *et al.*, 1995, Pflügers Arch. (abstract) in press; De Decker *et al.*, 1996 Abstract, Physiol. Soc. Meeting, Manchester, (to be published in J. Physiol.). If we consider an epithelium to consist of a cellular (R_{cell}) and a intercellular (or shunt) resistance, R_{sh} , in parallel, changes in R_{cell} will be reflected in significant alterations in R_{te} only if R_{cell} (i.e. the sum of the luminal, R_{lu} , and the basolateral membrane resistance, R_{bl}) is comparable to, or lower than R_{sh} . In the present study we investigated whether we could detect a change in R_{cell} with measurements of R_{te} . An isolated Px midgut was mounted in a luminal perfusion set-up. V_{te} was measured and current pulses were applied across the epithelium to calculate R_{te} using the appropriate cable equations (Boulpaep & Giebisch, 1978, In Martinez-Maldonado ed.: *Meth. in Pharmacology*, vol. 4b, 165-193, Plenum, London). Cells were impaled from the bath side and the membrane potential V_{bl} was measured. Voltage deflections caused by the current pulses allowed us to derive the voltage divider ratio, VDR, which is an estimate of the luminal, R_{lu} , over the basolateral membrane resistance, R_{bl} . If the basolateral membrane holds a K^+ conductance its resistance can be altered by changing the bath K^+ concentration and by applying the K^+ channel blocker, Ba^{++} , to the bath. In paired experiments a tenfold decrease of the bath K^+ reversibly hyperpolarized V_{bl} from -3 ± 1 to -17 ± 4 , $n=21$, $p < 0.01$, paired Student's t-test. This confirms the presence of a K^+ conductance in the cell membrane, as in most animal cells. At the same time R_{te} increased reversibly with about 10 % (from 139 ± 9 to 156 ± 11 , $n=21$, $p < 0.001$) and the VDR decreased with a factor of 2 (from 12 ± 1 to 7 ± 1 , $p < 0.02$). Lowering the bath K^+ and applying Ba^{++} (6 mM) at the same time is expected to cause even more pronounced changes: R_{te} increased reversibly with about 30 % (from 150 ± 9 to 200 ± 12 , $n=24$, $p < 0.001$) and VDR decreased with a factor of 3 (from 16 ± 2 to 5 ± 1 , $p < 0.001$). The data show that changes in membrane properties as reflected in VDR, provoke measurable changes in Px midgut R_{te} . The method can be used to detect δ endotoxin effects on luminal membrane resistance (De Decker *et al.*, 1996).

05-037

CRYIIIA EFFECTS ON AMINO ACID TRANSPORT

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Bacillus thuringiensis CryIIIA δ -endotoxin does not affect the transport of tyrosine and leucine into brush border membrane vesicles (BBMV) from midgut of a target insect, the Colorado potato beetle or jejunum of a non-target vertebrate, the rabbit, even at very high concentrations. The CryIIA toxin was six orders of magnitude more potent in inhibiting tyrosine transport into *Manduca sexta* BBMV than was the CryIIIA toxin. Both toxins eliminated the overshoot concentration of amino acid in *M. sexta* BBMV in the presence of an inward KSCN gradient. The physiology of the midgut transporters that leads to these results is discussed.

05-039

ACTIVATION OF THE CRYIIIA TOXIN OF *BACILLUS THURINGIENSIS* SUBSP. *TENEBRIONIS*: A COMPARATIVE ANALYSIS

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The pH values in the fore- and midgut of larvae of the Colorado potato beetle CPB (*Leptinotarsa decemlineata*) range between pH 4 and pH 7. The gut proteolytic enzymes have their highest proteolytic activity under moderately acidic pH conditions. Most of the proteases are cystein-dependent, no trypsin-like enzymes could be detected. The pH conditions in the midgut of the European cockchafer (*Melolontha spec.*) are neutral to alkaline (pH 7 to pH 9.5) with trypsin-like proteases as main proteolytic enzymes.

The crystal toxin of *Bacillus thuringiensis* subsp. *tenebrionis* is activated at acidic pH by CPB proteases. Treatment with gut fluid of the European cockchafer under alkaline conditions results in a 55 kDa toxin fragment which in bioassay is inactive against CPB.

Planar lipid bilayer (PLB) studies with CPB gut fluid activated CryIIIA reveal a channel with a main conductance level of 512 pS and a subconductance level of 128 pS under symmetrical conditions. The channel is selective for monovalent cations and voltage-independent. The channel formed by trypsin treated CryIIIA toxin has a main conductance level of 440 pS.

In order to assess the discrepancy between biological activity and channel formation in PLB experiments, a 38.8 kDa toxin fragment was produced by hydroxylamine cleavage covering residues 1 to 337 of the activated CryIIIA toxin and representing the proposed pore-forming domain I and a small portion of domain II (structure data according to Li *et al.* 1991). PLB analysis of this fragment revealed a channel with conductance levels between 116 pS and 270 pS under symmetrical conditions.

Li, J.; J. Carroll and D.J. Ellar. *Nature* 353, 815-821

05-040

MIDGUT MEMBRANE PORE FORMATION BY *BACILLUS THURINGIENSIS* INSECTICIDAL PROTEIN TOXINS

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The bacterium *Bacillus thuringiensis* produces specific insecticidal protein toxins (δ -endotoxins) grouped into two families - Cry and Cyt toxins - by sequence similarity. Both types of δ -endotoxin bind to insect-specific receptors on the surface of midgut epithelial cells and insert into the cell membrane to form leakage channels that result in cell death by colloid osmotic lysis. The X-ray structure of the first Cry toxin revealed putative membrane insertion and receptor binding domains whose functions have been explored by mutagenesis. The first Cyt toxin structure has now been described and is entirely different from the Cry toxins - despite their similar toxic mechanism. The first two Cry toxin receptors have recently been cloned and sequenced. These receptors are transmembrane proteins exposed on the lumen surface of midgut epithelial cells. Using *Manduca sexta* midgut brush border membrane vesicles (BBMV) we previously analysed the action of the δ -endotoxin Cry1Ac using a light-scattering assay (Carroll and Ellar 1993, *Eur. J. Biochem.* **214**, 771-778). This assay monitors vesicle volume changes and the effect of toxins by following the associated changes in scattered-light intensity as vesicles shrink and swell in an osmotic gradient. Activated Cry1Ac increased *M. sexta* BBMV membrane permeability for cations, anions and neutral solutes. However, vesicle volume is not the sole determinant of scattered light intensity. Solute-related refractive index changes and artefacts associated with vesicle motion and aggregation limit the quantitative application of this method, although it is still uniquely valuable in comparing different toxins under identical experimental conditions (Wolfersberger *et al.* 1996, *Appl. Environ. Microbiol.* **62**, 279-282). An extension of this approach based on the self-quenching of an entrapped fluorophore (Chen *et al.* 1988, *Biochemistry* **27**, 5713-5718) will be described. This allows quantitative comparisons of membrane permeability changes to different test solutes to be made. Changes in vesicle volume are followed by the resulting change in fluorescence of trapped carboxyfluorescein as the BBMV shrink and swell.

05-042

REGULATION OF TWO ECDYSONE RECEPTOR ISOFORMS AND ULTRASPIRACLE DURING *MANDUCA* DEVELOPMENTL. M. Riddiford, M. Jindra, M. Asahina, K. Hiruma, F. Malone
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During growth, molting, and metamorphosis of the tobacco hornworm, *Manduca sexta*, the developmental profiles in the epidermis for both mRNA and protein of the ecdysone receptor (EcR) and its heterodimeric partner Ultraspiracle (USP) differ. From the first instar on, USP is ubiquitously expressed with some fluctuations during the molts when a second transcript is also detected. After pupal commitment, USP mRNA remains high, whereas the protein transiently disappears from the nucleus, then reappears at the onset of pupal differentiation. During the adult molt, USP is highest at the onset of cuticle formation. By contrast, EcR levels are dependent on the ecdysteroid titer with EcR-B1 present at low levels throughout the intermolt periods of the 4th and 5th instars, then induced by low levels of 20-hydroxyecdysone (20E) ($ED_{50}=60$ ng/ml) at the onset of the larval, pupal and adult molts and at the time of pupal commitment. In the epidermis EcR-A is present at much lower levels except at the time of the peaks of the ecdysteroid titers for the larval and adult molts when it transiently appears as cuticle deposition begins. At the onset of metamorphosis during pupal commitment, EcR-A appears later than EcR-B1 consistent with its higher $ED_{50}=110$ ng/ml, then stays high through the onset of pupal differentiation. In contrast to *Drosophila* where EcR-A predominates at pupariation, both EcR-A and -B1 are high in *Manduca* wing discs during the onset of pupal development and show two peaks of expression during adult development with EcR-A persisting as the cuticle is being deposited. Thus, EcR-B1 is associated with maintenance and early predifferentiative events during a molt whereas EcR-A is primarily associated with the differentiative event of cuticle deposition, but also is involved with the switching that must occur in the polymorphic epidermis.

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05-041

Functional and phylogenetic studies of the pore formation domain from the *Bacillus thuringiensis* delta-endotoxins.

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Bacillus thuringiensis are Gram positive bacteria which produces proteinaceous inclusions during sporulation, these inclusion are composed of proteins known as insecticidal crystal proteins (ICPs) or δ -endotoxins, which are highly toxic, to a wide variety of important agricultural and health-related insect pests. A critical step for insecticidal activity is the binding of activated toxins to receptors located on the apical microvilli membrane of epithelial midgut cells (Hofmann *et al.*, 1988; Van Rie *et al.*, 1990a). The interaction of the toxin with those receptors triggers the opening of ionic channels (Sacchi *et al.*, 1986; Lorence *et al.*, 1995). These channels shunt the electrogenic pump potential difference and disrupt ion gradients and pH regulation, causing cell lysis, gut paralysis and death of the insect. The crystal structure of Cry3A (coleopteran specific) and Cry1Aa (lepidopteran specific) toxins have been reported (Li *et al.* 1991; Grochulski *et al.* 1995). Both toxins shares 36% amino acid identity and the two structures show high overall similarity. Both are globular molecules composed of three distinct domains connected by single linkers. Domain I has been implicated in the ion channel formation in the membrane. Domain II has been described as the specificity-determining domain. Domain III is proposed to stabilize the toxin by protection from proteolysis (Li *et al.* 1991). Experimental data from several laboratories have shown that domains from Cry proteins are structural independent. Isolation of domain I (Walters *et al.* 1993, von Tersch *et al.* 1994) and α -helix peptides (Cummings *et al.* 1993; Gazit *et al.* 1994) retains its ability to form cation channel formation in planar lipid bilayers. There are no reports of Domain II or Domain III isolation and expression, however exchanging sequence segments within domain II and domain III resulted in changes in specificity. These observations supports that Bt toxins are modular in structure and suggest that the different domains could have evolved independently. In this work we will present the characterization of the ion channel formed by Cry1Ac, Cry1C and Cry1D toxins in the presence and in the absence of their receptors. Our data shows that all toxins forms cation channels which are not highly selective among different monovalent cations, but they differ in their ionic conductances. We will show data regarding the analysis of mutants affected in alpha-helix 5, that are altered in the ion channel formation. Finally a phylogenetic relationship among the different domain I from all reported delta-endotoxins will be discussed.

05-043

DEVELOPMENTAL PROFILES OF EcR EXPRESSION DURING WING MORPHOGENESIS IN *BOMBYX MORI*.H. Fujiwara and M. Kamimura¹Department of Biological Sciences, University of Tokyo,
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The wing disc of insects changes dramatically in size and shape during metamorphosis and thus is a good model to study the mechanism of tissue differentiation. The metamorphic processes are triggered by ecdysone, which actions are mediated by the ecdysone receptor (EcR) to control the gene expression in the target tissues. We identified the ecdysone receptor gene (BmEcR) and studied its mRNA expression during wing formation in *Bombyx mori*. The expression of B1 form of the BmEcR was induced at day 4-5th, maximal on the day of wandering and repressed during pharate pupa. The ecdysteroid titer, the EcR expression pattern and the morphological changes seem to be closely related. The expression pattern of EcR in wing discs is also similar in *Manduca sexta*, suggesting that the temporal pattern is essential to form the wings during metamorphosis among lepidopteran insects. This idea has been further supported by an observation that the mRNAs of EcR B1 and A form are not normally expressed in the wing discs of a wingless mutant, fl (flügellos) of the silkworm. We will discuss about the role of the EcR on the wing morphogenesis through comparison between *Bombyx* and *Manduca* system and between the wild type and fl mutant of the silkworm.

05-044

STEROID RECEPTOR SUPERFAMILY GENES AND THEIR RELEVANCE TO DIAPAUSE AND EMBRYOGENESIS OF *BOMBYX MORI*

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With onset of embryonic diapause of *Bombyx mori*, glycogen converts into sorbitol and glycerol. At diapause termination which is caused by cold-acclimation, an utilization of sorbitol for re-synthesis of glycogen is controlled by sorbitol dehydrogenase, of which gene expression is induced by cold acclimation. Further to understand molecular mechanism how cold temperature works on the gene for sorbitol dehydrogenase, the gene structure was analyzed. Some nucleotide sequences similar to cis-elements which are recognized and bound by steroid receptor superfamily members such as COUP-TF/Seven up, FTZ-F1 and DHR39, were found in 5'-up stream region of the gene for sorbitol dehydrogenase. cDNAs for *Bombyx* homologs of Seven up, DHR 39 and EcR were cloned and sequenced, and the amounts in mRNAs for Seven up, BHR 39, FTZ-F1 and EcR were examined in eggs during diapause and embryogenesis. Especially, the profiles of gene expressions for *Bombyx* homologs of Seven up and EcR are discussed in relation to the gene expression for sorbitol dehydrogenase during diapause and embryogenesis.

05-046

PARTIAL CHARACTERISATION OF AN ECDYSTEROID RECEPTOR GENE FROM THE APHID *MYZUS PERSICAE*
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Aphids have been refractory to classical methods of endocrinological investigation because of their small size. Their polyphenism (winged or wingless, parthenogenetic or sexual) and their very rapid viviparous parthenogenetic reproduction have long been suspected of being under control of ecdysone and juvenile hormone. There is increasing evidence from other insects that spatial and temporal ecdysteroid receptor expression is related to metamorphosis (and by extrapolation, to polyphenism). We have prepared cDNA libraries from third instar *Myzus persicae*, a cosmopolitan aphid which serves as vector of viral diseases to many economically important crops. The libraries were probed initially with sequence for the DNA-binding domain from the *Drosophila* ecdysteroid receptor gene (*DmEcR*), and subsequently with a probe derived by PCR from *M. persicae* genomic DNA. We have located, cloned and sequenced from our random-primed library a 700 bp fragment with many features suggesting that it is part of an aphid *EcR*. It contains a region coding for a DNA-binding domain and exhibits 86-88% identity, and 91-95% similarity, with other known *EcRs*. The initial fragment does not include the region coding for the hormone-binding domain, and a full-length insert is being sought in the oligo-dT primed library. The complete sequence will enable us to commence expression studies, and eventually to study the spatial and temporal distribution of *EcRs* in aphids in relation to their unusual biology.

05-045

MOLECULAR ANALYSIS OF ECDYSTEROID ACTION DURING MOSQUITO VITELLOGENESIS
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In mosquitoes, 20-hydroxyecdysone (20E) plays a critical role in regulation of vitellogenic events in the fat body and ovaries. To elucidate the ecdysteroid regulatory cascade controlling mosquito vitellogenesis, we cloned the cDNAs encoding the *Aedes aegypti* ecdysteroid receptor (AaEcR) and its heterodimeric partner, the Ultraspiracle homolog (AaUSP). Two major transcripts of AaEcR (4.2 and 6 kb) are expressed during the previtellogenic and early vitellogenic period in both the fat body and ovaries. Four AaUSP transcripts, 1.8, 2.2, 2.5, and 3.0 kb, are expressed in these vitellogenic tissues with the smaller transcripts predominating in the ovary and the 3.0-kb transcript in the fat body. Two cloned AaUSP isoforms differ in their A/B domains. Both AaUSP isoforms form active heterodimers with AaEcR which bind *Drosophila* hsp27EcRE. 10⁻⁸ M 20E is required for maximal DNA binding activity of AaEcR/AaUSP. Experiments utilizing 20E and cycloheximide showed that the action of 20E on the target vitellogenic genes, vitellogenin and vitellogenic carboxypeptidase, is indirect. Therefore, we have begun the search for the early genes mediating 20E response in the mosquito by cloning the *Drosophila* E75 homolog (AaE75). Analysis of the AaE75 gene and cDNAs showed that three isoforms (A, B, and C) result from alternative exon usage and are expressed in both the ovary and fat body during vitellogenesis.

05-047

MOLECULAR BASIS FOR SELECTIVE INSECT TOXICITY OF THE NON-STEROIDAL ECDYSTEROID AGONIST, RH 5992

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RH 5992 (Tebufenozide, MIMIC[®], CONFIRM[®]), the first non-steroidal ecdysteroid agonist undergoing worldwide commercial development, manifests its toxicity in lepidopteran larval pests via interaction with the ecdysteroid receptors (EcR). Interestingly, in spite of the fact that most insects use 20-hydroxyecdysone (20E) as the molting hormone, the action of which is mediated via the ecdysteroid receptors, RH 5992 is toxic only to lepidopteran larvae. Our previous experiments to explain this selective toxicity of RH 5992 indicated that there were no differences in the transport and metabolism of RH 5992 in susceptible lepidopteran and non-susceptible coleopteran insects tested. We have carried out additional experiments to understand the molecular basis of the selective toxicity of RH 5992. Although, RH 5992 binds to various EcRs in cellular extracts or to EcR/ultraspiracle (USP) proteins produced *in vitro*, it does so with significantly different affinities. Ligand-induced changes in EcRs can be visualised by subjecting radiolabeled EcR in ligand-EcR-USP complexes to partial proteolysis followed by analysis of the digests by SDS-PAGE and fluorography. Results of such experiments with Muriesterone A and RH 5992 and one lepidopteran and two dipteran EcRs revealed differences in RH 5992 induced conformational changes in different EcRs. Muriesterone A induced conformational changes in all the three EcRs. The significance of these results will be discussed with respect to the selective toxicity of RH 5992 in insects.

05-048

LEPIDOPTERAN AND DIPTERAN 20-HYDROXY-ECDYSONE RECEPTORS: STRUCTURAL AND FUNCTIONAL SIMILARITIES AND DIFFERENCES

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The *Drosophila* ecdysone receptor (DmEcR) functions as an obligate heterodimer with another nuclear receptor, DmUSP. Both EcR and USP homologs have been cloned in the domesticated silkworm, *Bombyx mori*. Their sequences differ considerably from those of their *Drosophila* counterparts; only within their DNA-binding domain do the receptors show high levels of sequence conservation. Because the sequence changes in the ligand-binding and transactivation domains of the receptors may reflect functional differences, we have begun testing whether *Drosophila* and *Bombyx* receptor components are interchangeable.

Our data indicate that the components of a functional ecdysone receptor complex are conserved among Diptera and Lepidoptera. Upon dimerization with BmCF1 (the silkworm homolog of USP), BmEcR binds ¹²⁵I-iodoponasterone A with a K_d of 1.1 nM, and forms a specific, high affinity complex with a *Drosophila* ecdysone-response element (EcRE). BmEcR can also substitute for DmEcR in an EcR-deficient *Drosophila* tissue culture line, stimulating transactivation of an ecdysone-inducible reporter gene construct.

However, significant differences were observed between DmEcR and BmEcR in their capacity to induce ecdysone-dependent gene expression in a *Drosophila* cell line that is deficient in endogenous EcR; gel retardation assays also revealed that BmEcR/DmUSP heterodimers bind more stably to the *Drosophila* EcRE than BmEcR/BmCF1 ones, reflecting differences in the dimerization properties of Dipteran and Lepidopteran receptors and differences in the stability of complexes formed between the two heterodimers on the *Drosophila* EcRE. Thus, it appears that despite the functional equivalence of the ecdysone receptor components, differences do exist with respect to the ecdysone response between Lepidoptera and Diptera.

We are currently conducting additional binding assays to determine the stability of complexes formed between DmEcR/BmCF1 heterodimers and the *Drosophila* EcRE. Work is also in progress to isolate EcRE sequences from lepidopteran (*Bombyx*) ecdysone early response genes.

05-050

CROSS-TALKING AMONG *DROSOPHILA* NUCLEAR RECEPTORS AT THE PROMISCUOUS HORMONE RESPONSE ELEMENT OF THE *ng-1* INTERMOULT GENE

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In *D. melanogaster*, three temporally distinct ecdysone-responsive puff sets, the so-called intermolt, early and late puffs, have been described on the salivary gland polytene chromosomes. The group of "intermolt" puffs is the first that is transcriptionally active during the third instar. Intermolt genes encode salivary gland-specific proteins, so that their expression is part of the gland-specific pre-metamorphic response to ecdysone. We are studying the early hormonal response by focusing on *ng-1* and *ng-2*, a pair of highly homologous genes mapping at the 3C intermolt puff. The *ng*-genes start to be transcribed at the beginning of the third instar, when the ecdysteroid titer is still low, to be repressed at the onset of the wandering phase, in concomitance with the induction of the other intermolt genes. This peculiar developmental expression profile provides an unique opportunity to investigate the hormonal regulatory hierarchy at a developmental window not widely explored. Two EcREs of identical sequence have been identified within the coding region of the *ng-1* and *ng-2* intermolt genes (D'Avino et al., Mol. Cell. Endocrinol. 113:1-9, 1995). These EcREs have now been characterized at the molecular and functional level. The ability of binding the EcR/USP heterodimer has been shown to depend on two directly repeated half-sites separated by 11bp (DR11). As structurally analogous vertebrate HREs composed of widely spaced direct repeats, the *ng*-EcREs act as promiscuous response elements able to bind several *Drosophila* nuclear receptors, thus offering a good experimental model system to studying receptor cross-talking. At the functional level, transient expression and P-element mediated transformation experiments show that the *ng*-EcREs act as enhancers to direct high-level *ng-1* and *ng-2* expression.

05-049

MODULATION OF HORMONE AND DNA BINDING BY BmCF1 A LEPIDOPTERAN HOMOLOGUE OF *ultraspiracle*, PARTNER OF ECDYSONE RECEPTOR AND THE NGFI-B INSECT HOMOLOGUE BHR38George Tzertzinis^{1,2}, Tarlochan S. Dhaddiala², Tatjana Kozlova¹, James Sutherland¹ and Fotis C. Kafatos¹
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BmCF1 is a *Bombyx mori* homologue of the *Drosophila* *ultraspiracle* (USP), having greater than 95% identical amino acid residues in the zinc finger domain and 50% in the ligand domain. BmCF1, like USP but unlike its mammalian homologue RXR, does not show any hormone or DNA binding activity alone, but it readily heterodimerises with EcRs to bind both hormone and DNA targets. We studied the properties of homospecific (*Drosophila*) and heterospecific (*Drosophila/Bombyx*) EcR/USP heterodimers with respect to their hormone and DNA binding properties.

Comparison of the affinities of ecdysteroids to DmEcR/BmCF1 and DmEcR/USP suggests that BmCF1 can substitute for USP in modulating ecdysteroid binding by EcR. Surprisingly though, its strongly conserved DNA-binding zinc finger domain seems to display different DNA binding properties and thus affect the specificity of the formed heterodimers.

Recently we isolated a new partner of USP, hormone receptor 38 (DHR38 and BHR38 from *Drosophila* and *Bombyx* respectively) a homologue of the mammalian NGFI-B type receptors which were originally thought to function exclusively as monomers.

Both *Bombyx* and *Drosophila* HR38 heterodimerise avidly with BmCF1/USP constituting an additional to EcR partner of USP. The strong binding of HR38 to BmCF1 and USP precludes USP/EcR heterodimerisation and consequently, ecdysone response. The precise role of HR38 in the ecdysone response mechanism is under current study.

05-051

ACCESSIBILITY OF ECDYSONE RECEPTOR BINDING SITES IN *CHIRONOMUS* POLYTENE CHROMOSOMESM. Lezzi, C. Elke, M. Vögeli, F. Gatzka, P. Rauch¹, M. Spindler-Barth¹, K.-D. Spindler¹Institut für Zellbiologie, ETH, Zürich, Switzerland - ¹ Lehrstuhl für Hormon- und Entwicklungsphysiologie, Heinrich-Heine-Universität, Düsseldorf, Germany

The ecdysone receptor (EcR) is a transcription factor whose functioning depends not only on the nuclear concentration level of itself and of its heterodimerization partner USP but also on the cellular titer of its ligand (i.e. ecdysteroids, Ec) as well as on the accessibility of cognate response elements in DNA (EcREs) flanking Ec-responsive genes. In situ studies with polytene chromosomes of larval salivary glands of *Chironomus tentans* suggested a model in which the state of chromatin condensation would control access of Ec-activated EcR/USP complexes to EcREs in specific chromosomal loci. In order to test this model the genes coding for EcR and USP of *C. tentans* were cloned and over-expressed in *E. coli* cells. The affinity purified cEcR and cUSP molecules exhibited the expected binding specificity for active EcREs and Ec in vitro. The bacterially produced and purified nuclear hormone receptor proteins were incubated in the presence of Ec with native isolated polytene chromosomes of a stage with a very low titer and minimal chromosomal localization of the endogenous receptors. The exogenously added cEcR accumulated preferentially at chromosome regions which are always slightly decondensed and Ec-responsive while leaving unoccupied those chromosome regions which in vivo decondense and accommodate endogenous EcR under certain physiological conditions only.

05-052

THE ASSEMBLY OF THE VARIOUS INTERCELLULAR JUNCTIONS IN INSECT TISSUES

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The tissues in insects maintain their integrity by the associations between their component cells. Cellular adhaering molecules (CAMs) help to maintain these intercellular contacts and it seems likely that cadherins are initially responsible for adjacent cells coming into alignment. The assembly of the intramembranous junctional components must then follow. This is complicated by the additional necessity for the extracellular moities to establish contact with one another and for the associated cytoskeletal components to make the appropriate links to those transmembrane molecules that are specifically characteristic of the junction. The situation is further complicated by the fact that there are extensive junctional complexes along the lateral borders of cells both in epithelial layers and in other tissues. Thus, more than one junction may be found on any one border and the molecular constituents of these may be interwoven one with another in an intricate fashion. Junctional types comprising these complexes include septate, scalariform, gap, tight and adhaering junctions, with different tissucs exhibiting varying combinations of these. The distinct features of each of them, their differing molecular nature, organization and associated cytoplasmic proteins, makes it the more astonishing that their assembly is so free from error.

05-054

JUNCTIONAL PROTEINS AND CELL PROLIFERATION CONTROL IN DROSOPHILA

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ABSTRACT NOT RECEIVED

05-053

SEPTATE JUNCTIONS AND INTERCELLULAR ADHERING STRUCTURES IN INSECTS.

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Septate junctions (pleated and smooth types) are the commonest models of junctions present between insect epithelial cells (Lane et al 1994). Midguts of the grasshopper *Pezotettix* and of *Tenebrio* mature larvae prepared by the quick-freeze, deep-etching technique revealed bundles of actin filaments and microtubules in close vicinity of the plasma membrane at the junctional level. A network of strands connects with one another microfilament bundles descending from microvilli. Many of these strands have the same diameter and repeat of actin filaments; some others have a thinner section and no repeat. In the collembolan *Isotomurus* the region beneath microvilli shows a conspicuous filamentous web which receives the bundles of microfilaments of microvilli and is anchored to the plasma membrane at junctional level (Dallai et al. 1993). Quick-freeze, deep-etching microscopy allowed to visualize two classes of filaments in the web: one is in continuity with the microfilaments of the bundles; a second type of filaments thinner than actin filaments, takes contact with the plasma membrane.

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05-055

STRUCTURAL AND GENETIC ANALYSIS OF DUCTIN IN DROSOPHILA AND CRUSTACEA; A COMPONENT OF THE GAP JUNCTIONS AND VACUOLAR H⁺-ATPase

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Ductin is the highest conserved polytopic membrane protein yet known having 80% identity between fungi and *Drosophila*. It forms the site of proton translocation in the vacuolar H⁺-ATPase (V-ATPase) which is the primary proton pump of endomembranes and the plasma membrane of some cell types, most notably certain epithelial cells of the insect midgut and malpighian tubules. In addition, in metazoan animals, ductin is the structural subunit of the connexon of gap junctions. These intercellular specialisations are thought to be the sites of cell-cell communication by allowing the cell-to-cell movement of low molecular weight solutes. Gap junctions containing ductin can be isolated in bulk from the hepatopancreas of the Norway lobster and this, together with a yeast expression system, has allowed a detailed analysis of the structure of ductin and the complex it forms. Ductin forms a four helix bundle in the membrane and six ductin molecules constitute the connexon of the gap junction or the membrane domain of the V-ATPase. The first transmembrane helix lines the central channel of the connexon, whilst the fourth helix is the principal site of proton translocation. In *Drosophila* there is a single gene on chromosome 2R which gives rise to a single product. P-element insertion has been used as a strategy to inactivate the ductin gene (*vha16*). The second intron is a hot spot for P-element insertion indicating a high transcriptional activity of the gene. Homozygous flies containing a tagged P-element die in embryonic stages suggesting loss of the *vha16* gene function is lethal. Heterozygous flies though, appear quite normal.

05-056

NUTRITIONAL REQUIREMENTS OF INSECT CELLS CULTURED IN VITRO

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 Since T.D.C. Grace established the first insect cell line in 1962, more than 200 cell lines have been established. Some have recently been used intensively for the production of recombinant proteins by means of the vaculovirus vector system. In order to produce such useful proteins on a commercial basis, it is necessary to develop culture media which support large-scale insect cell cultures. Elucidation of the nutritional requirements of insect cells will aid the development of effective culture media.

In, general, insect cell culture media consist of inorganic salts, sugars, amino acids, vitamins and some growth promoting substances. Insect cells are known to tolerate a wide range of pH and osmotic pressure. NaCl is not essential, and many cell lines can grow in media of various inorganic compositions. As energy source, glucose alone is usually sufficient. The amino acids Arg, Cys, Glu, His, I-leu, Leu, Lys, Met, Phe, Pro, Ser, Thr, Try, Tyr, and Val are essential for most insect cell lines, while the only vitamins required are thiamine, riboflavin, pantothenate and niacin. Many insect cell lines require a growth promoting substance(s) such as fetal bovine serum for their growth, while some can grow in serum-free media.

05-058

THE STRUCTURAL CHANGES OF THE MALE REPRODUCTIVE ORGANS DURING THE REPRODUCTIVE DIAPAUSE AND ITS TERMINATION IN *BRUCHIDIUS ATROLINEATUS* (PIC) COLEOPTERA BRUCHIDAE.J. Huignard, I.A. Glitho¹, A. Lenga

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The secretions of the male accessory glands play an important role in bruchid reproduction as demonstrated the study of the male reproductive diapause. These glands were non functional during diapause and the first phase of the diapause termination was the accumulation of secretions in the lumen of the gland. In the tropical bruchid, *Bruchidius atrolineatus*, the diapause was induced during post-embryonic development and its intensity depended on the conditions of induction. The diapause intensity was high when diapause was induced in conditions of 23:16°C 12-12h, 13:11h LD, 20-30% rh. There was no spermatogenesis in the tests and the secretory activity of the male accessory glandular cells was limited as long as the males remained in these climatic conditions. The diapause terminated only when the beetles were exposed to shorter photoperiod (11:13h LD), higher humidity (70-80% rh) and to the presence of host plant cues. The diapause intensity was low when diapause was induced in conditions of 40:25°C 12:12h 13:11h LD, 20-30% rh. The secretory activity of the cells of the accessory glands progressively increased and diapause spontaneously terminated when the secretions accumulated in the lumen of the glands. During this phase of diapause termination, the males exhibited a sexual behaviour and deposited during mating a spermatophore in the bursa copulatrix of the females.

05-057

INSECT CELL CULTURES: RETROSPECT AND PROSPECT.

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A brief account of the early pioneering work on insect cell and tissue culture will be presented, starting with Goldschmidt's 1915 attempts to maintain silkworm sperm in vitro. The successful cultivation of viruses in insect cells by Trager and improvements of culture media by him and Silver and Wyatt, leading to the first cell lines by Gao in China and Grace in Australia will be highlighted. Applications of insect cell cultures in genetics, toxicology and biological control of vectors and pests will be described. Current biotechnology applications, including vaccine and drug production, development of cost-effective media and of chemically defined media will be presented.

Predictions for the future will be based on expected solving of several technical problems and on cost-effective large scale industrial production methods. The next decades will most likely witness a large increase in biotechnological applications of cultured insect cells. Novel biotechnological strategies including recombinant DNA and insect cell fusion technology hold considerable promise for the future. It is also conceivable that cost-effective large scale insect cell production will be used to supplement traditional sources of animal and human nutrition.

05-059

PHYSIOLOGICAL ROLES OF THE DROSOPHILA SEX-PEPTIDE: A PEPTIDE PHEROMONE OF THE MALE ACCESSORY GLAND

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Mating provokes two well defined reactions in the female of *Drosophila* and many other insects: receptivity is reduced and production of eggs increased. In *D. melanogaster*, products of the male reproductive system transferred to the female during copulation, are a primary cause for these effects. Sex-peptide (SP), a peptide of 36aa, plays a major role in the induction of the two post-mating responses. SP is sufficient to elicit the two reactions, but it is not known whether it is also necessary.

Injection of synthetic SP, or ectopic expression in a virgin female, elicit the post-mating responses, suggesting that the SP finds its target(s) via hemolymph. A single kind of target molecule might be responsible for both reactions, however, more than one target tissue is involved.

After a normal mating the two post-mating responses last 7-9 days. In contrast, injection of physiological amounts of SP elicit the reactions for about 1 day. Males which do not transfer sperm, but normal amounts of accessory gland substances, induce egg-laying for about 2 days. Thus, sperm seems to play a role in the persistence of the two reactions. Transgenic flies containing a *yp1* enhancer fused to the SP gene express SP constitutively in the fat body of the adult female and secrete it into the hemolymph. These females permanently lay eggs at the high rate of mated females and show reduced receptivity. These results suggest, that SP is sufficient to elicit the two post-mating responses and that sperm might function as a "stabilizer" for the sex-peptide.

05-060

CONTROL OF SPERM DIMORPHISM IN LEPIDOPTERA

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Lepidopteran spermatogenesis is dichotomous and consists of (a) regular eupyrene spermatogenesis leading to the generalized type of insect spermatozoa and (b) irregular apyrene spermatogenesis leading to anucleate spermatozoa. Both kinds of spermatozoa reach the spermatheca but only the eupyrene one fertilizes the eggs. Both types of sperm derive from the same kind of bipotential early primary spermatocytes which differentiate into either eupyrene or apyrene cells. Under non-experimental conditions, the timetable of the dichotomous spermatogenesis is very strict and correlated with the status of ontogenetic development of the individual, and characteristic for a given species. Usually, eupyrene spermatogenesis begins in the larva and stops in the pupa while apyrene spermatogenesis begins just either before or after pupation, according to the species, and continues in the imago. The switchover of spermatocyte commitment is regulated by a haemolymph factor becoming active close to pupation. Spermatogenesis is a discontinuous process punctuated by predetermined stations. Progress from one station to the next is under hormonal control as shown experimentally by (a) transplantations and extirpations of endocrine glands and testes, (b) ligatures, (c) cultures of testes, and (d) treatments by a non-ecdysteroidal ecdysone-agonist, 20-hydroxyecdysone, juvenile hormone, and juvenoids.

Fluctuations of the endocrine balance control the progress of meiosis and sperm differentiation, the stoppage and renewal of spermatogenesis related to diapause, and the shift of spermatogenesis commitment from eupyrene to apyrene development.

05-062

INITIATORIN, A SERINE-ENDOPEPTIDASE AS INDUCER OF SPERM MATURATION OF *BOMBYX MORI* (L.) (LEPIDOPTERA: BOMBYCIDAE)

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Like other Lepidoptera, *Bombyx mori* produces two types of spermatozoa, denucleated apyrene sperm and nucleated eupyrene sperm that fertilize eggs. These two types of spermatozoa accumulate in the vesicula seminalis without motility. In this organ, apyrene sperm are separated, while 256 eupyrene sperm are packed in the cyst as a bundle. Initiatorin which is a serine-type endopeptidase with 30KDa secreted from the male glandula prostatica acts as the apyrene sperm-activating factor as well as eupyrene sperm bundle-dissociating factor in the spermatophore of females. These two physiological functions of initiatorin were confirmed by the purified specimen obtained using an affinity-chromatography with a column fixed with its inhibitor, p-aminobenzamidine, as a legend. This enzyme has more two capacities to initiate the arginine degradation cascade, an energy-yielding system for sperm maturation by hydrolyzing arginine-rich proteins and to activate the zymogen of an arginine-carboxypeptidase. These reactions for sperm maturation occur in the spermatophore of *Bombyx* silkworm: namely, this organ acts as a reactor of various metabolic reactions for sperm maturation. Trypsin used as a model of initiatorin induced the acquisition of motility of eupyrene sperm of Orthoptera as well as that of apyrene sperm of Lepidoptera. Heterogeneous cross-reaction of immotile apyrene sperm in the vesicula seminalis and the prostatic secretion between two different lepidopteran species caused motility of apyrene spermatozoa.

05-061

CIRCADIAN RHYTHM OF SPERM RELEASE FROM TESTIS IN MOTH: ULTRASTRUCTURAL STUDY

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In insects, mature bundles of elongated spermatozoa leave the testes prior to the reproductive stage. Release of sperm bundles from testis in moths is rhythmic and controlled by a circadian clock which is located in testis-vas deferens complex. Sperm bundles are released from the fused testis into the paired upper vasa deferentia (UVD), then transferred into the seminal vesicles in a two-step rhythm controlled by light-dark cycles (LD). In LD, 16:8, sperm bundles are released from the testis only during the 4 h release gate in the evening, remain in the UVD overnight and are transferred to seminal vesicles in the morning. Rhythmic release of sperm was studied in detail in the gypsy moth, *Lymantria dispar*, using Scanning and Transmission Electron Microscopy. To be released from the testis, sperm bundles must penetrate through the basilar membrane - a layer of specialized epithelial cells separating testis follicles from the UVD lumen. Through most of the day, sperm bundles remain in contact with the testis side of basilar membrane but no penetration of bundles occurs. Approximately 3 h before sperm is first seen in the UVD, sperm bundles, still enclosed in cyst cells, start to penetrate between the cells of basilar membrane. Soon thereafter, sperm bundles dissociate from cyst cells and rapidly translocate into the UVD lumen. Subsequently, cyst cells become fragmented and degenerate.

05-063

HISTOPHYSIOLOGY OF MALE ACCESSORY GLANDS OF *DYSDERCUS KOENIGII* F. SEQUENTIAL TO ITS DEVELOPMENTS.S. Sehgal, K.K. Gupta¹Department of Zoology, University of Delhi, Delhi-7. ¹Department of Zoology Deshbandhu College (University of Delhi), Kalkaji, New Delhi-19 India.

The male accessory glands of *Dysdercus koenigii* develop as terminal evaginations of vasa deferentia in early nymphal instars. Each evagination elongates and ultimately thrown into several loops. Histologically, in a newly emerged adult the gland consists of tall columnar epithelial cells that fill the interior of tubule completely. Subsequently a transition of these cells from columnar to cuboidal results in formation of a spacious lumen which later on get filled with some secretions. The secretory activity commences after 24 hr of emergence. The secretions are completely transferred to female during mating. Histochemical studies have indicated an age related difference in the distribution and localization of proteins, lipids, glycogen and nucleic acids within the glandular epithelium and in the secretions. SDS PAGE electrophoresis of the accessory glands of sexually mature male resolved 35 protein bands accounting both structural and secretory proteins. Specific staining with Sudan black B and PAS reagent indicated lipoprotein and glycoprotein nature of a few protein bands. Secretory proteins were mainly of low molecular weight ranging from 14200-29000, which showed variations during development, sexual maturation and mating in the adult.

05-064

8. GENETICS AND EVOLUTIONARY ENTOMOLOGY
J.H. WERREN AND R. STOUTHAMER. ORGANIZERS

ALTERATION OF SPERM PROTEINS BY *WOLBACHIA*: A MOLECULAR MODEL FOR CYTOPLASMIC INCOMPATIBILITY
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In *Drosophila simulans* as in many other insect species, a unique form of male sterility known as cytoplasmic incompatibility (CI) is mediated by an endocellular bacterium, *Wolbachia*. This bacterium reduces or abolishes the ability of males to father offspring with uninfected females. From the perspective of *Wolbachia*, CI effectively spreads and maintains the bacteria by preventing the production of uninfected host offspring whenever the male parent is infected. *Wolbachia* readily cross from the ovariole into developing ova, resulting in nearly 100% maternal transmission. By contrast, the exclusion of cytoplasm from the developing sperm usually results in the complete absence of *Wolbachia* in mature sperm. Accordingly, CI cannot be due to sperm-borne *Wolbachia*, and therefore the bacterium must have exerted its influence earlier during spermatogenesis.

Sperm from males infected with *Wolbachia* contain reduced and/or altered protein profiles. Anti-*Wolbachia* monoclonal antibodies were found to recognize cellular host proteins suggesting that *Wolbachia* exert their influence by reducing the levels of specific proteins in the mature sperm. This hypothesis has been confirmed by 2D gel electrophoresis of sperm from infected and uninfected males. Two major and two minor regions have been identified on 2D gels. The two major regions occur in the 70-90 kilodalton range. These proteins are immunologically related to Hsp90 and Hsp70 family as confirmed by immunoblots using anti-hsp90 and anti-hsp70 antibodies. Hsp90/hsp70 have been implicated in numerous signal transduction processes suggesting a model- where *Wolbachia* remove specific isoforms of hsp90 necessary for proper egg activation.

05-066

IS THERE A SINGLE Na^+/K^+ -ATPASE α -SUBUNIT IN INSECTS?

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The Na^+/K^+ -ATPase (sodium/potassium pump) translocates sodium and potassium ions across the plasma membrane of animal cells. The enzyme is widespread in vertebrate cells where the electrochemical gradients generated by at least three different isoforms are essential for many secondary transport processes, as well as renal and neuronal functions. In insects, the sodium/potassium pump may be more restricted, especially among the Lepidoptera, where the enzyme is apparently absent from all but nerve tissue.

Multiple molecular forms of the sodium/potassium pump α -subunit can be distinguished as doublets in SDS-PAGE. Our results with *Anopheles stephensi* and *Stomoxys calcitrans* are consistent with a single molecular form of the α -subunit which is found predominantly in the head, with lesser concentrations in the midgut, Malpighian tubules and thorax. Biochemical analysis of the midgut and head enzyme using the specific cardiotonic steroid, ouabain, indicate no apparent differences between the two tissues.

Using a PCR approach we have compared partial sequences of insect Na^+/K^+ -ATPases, and concluded there is likely to be a single gene for the α -subunit of this enzyme in Diptera and Lepidoptera, whereas vertebrates have at least three distinct genes (Emery *et al.* 1995). The insect form is apparently distinct from any one of the vertebrate isoform classes.

Emery *et al.* (1995). *Insect Molecular Biology* 4:179-192

05-065

MALE SPECIFIC PROTEIN IN THE HEMOLYMPH OF GREATER WAX MOTH, *GALLERIA MELLONELLA* L.

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Male specific protein(MSP) was identified and purified from adult male hemolymph of *Galleria mellonella* L. by gel electrophoresis and anion exchange chromatography. Its physiochemical property including molecular weight, amino acid composition and carbohydrate were also investigated. The presence of MSP in various organs was confirmed during developmental stages by western blotting and immunodiffusion using antibody against it. MSP was present in fat body as well as hemolymph of adult male. MSP is also present in testes of late pupae and adults. Fat body of pupae and adults was *in vitro* tissue-cultured in culture medium including [³⁵S]-methionine. MSP was actively synthesized in fat body of adult male. Function and fate of MSP were also being investigated.

05-067

EXPRESSION OF A MIDGUT AMINO ACID TRANSPORT SYSTEM IN *XENOPUS* OOCYTES

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In the last years the functional expression cloning strategy led to discover the structure of many proteins involved in membrane transport processes. The first step of this experimental approach is the expression of a new protein after injection of exogen mRNA in *Xenopus* oocytes. In this study, mRNA purified from midguts of *Philosamia cynthia* larvae was injected into *Xenopus laevis* oocytes and the increased leucine uptake was interpreted as expression of a new transport system whose features closely resemble those of the amino acid transport system present in the tissues where mRNA was purified from. Amino acid transport in the midgut of several species of lepidopteran larvae involves a new class of amino acid cotransporters with broad specificity for the driver cation which can be potassium, sodium and in some case lithium. Sodium affinity for the transporter is about 18 times that of potassium, whereas, at saturating cation concentrations, leucine transport rate was 2.5 times higher with potassium. Sodium activation curve of leucine uptake was sigmoidal in control oocytes and hyperbolic in mRNA-injected oocytes. The difference between the two curves gives the sodium activation of the newly expressed leucine transport. The mean K_{50} was 0.4 ± 0.24 mM, a value near to that found in *P. cynthia* larvae and about 50 times lower than the K_{50} value measured in control oocytes. mRNA from *P. cynthia* midgut was fractionated on sucrose density gradient and after injection of fractions, two pools could be identified which caused an increase of leucine uptake.

05-068

UNUSUAL HIGH CONCENTRATION OF FREE GLYCINE IN THE MIDGUT CONTENT OF THE SILKWORM, *BOMBYX MORI*, AND OTHER LEPIDOPTERAN LARVAE

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In the course of investigating the digestion and the absorption of dietary protein by the silkworm, *Bombyx mori*, reared on an artificial diet, we found the existence of an unusually higher concentration of free glycine in the midgut content compared with other amino acids. To test whether this free glycine is from diet or from the silkworm itself, silkworm larvae were fed a diet without a protein source for 1 day and the free amino acids in the midgut content were then analyzed. Unexpectedly, the concentration of free glycine in the midgut content was still high (8.98 μ moles/g midgut content). In contrast, the concentrations of other amino acids were very low, and none of them exceeded 1/20 that of glycine. The concentration of glycine was high in the anterior half of the midgut and it decreased toward the posterior region. Free glycine was virtually undetected in the feces. A similar phenomenon, as in the silkworm, was observed in the sweet potato hornworm, *Agrius convolvuli*, but not in the rice armyworm, *Pseudaletia separata*. We also found that an unusual high concentration of free glycine exist in the digestive juice of *Bombyx mori*.

These results suggest that free glycine is secreted into the intestinal lumen for some biological purpose.

05-070

JUVENILE HORMONE AND JUVENILE HORMONE ACID BIOSYNTHESIS BY THE CORPORA ALLATA OF ADULT FEMALE AND MALE LOREYI LEAFWORM, *MYTHIMNA LOREYI* DUPONCHEL (LEPIDOPTERA: NOCTUIDAE)

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In *Mythimna loreyi*, the *in vitro* releases of juvenile hormone II (JH II) and juvenile hormone III (JH III) by adult female corpora allata (CA) were age-dependent, which peaked at day 4 (1.19 and 2.92 pmol/ 4h/ pair CA for JH II and JH III, respectively) and day 9 of adulthood (2.43 and 4.95 pmol / 4h/ pair CA for JH II and JH III, respectively). The *in vitro* releases of juvenile hormone acid I (JHA I), Iso-juvenile hormone acid II (Iso-JHA II) and juvenile hormone acid III (JHA III) by adult male CA were also age-dependent; the trends of JHA I and Iso-JHA II were similar, which peaked at day 3 (11.56 and 9.64 X 10² pmol/ 24 h/ pair CA for JHA I and Iso-JHA II, respectively) and day 10 (6.46 and 10.71 X 10² pmol/ 24 h/ pair CA for JHA I and Iso-JHA II, respectively); for JHA III, the release rate peaked at day 4 (18.42 X 10² pmol/ 24 h/ pair CA) and day 10 (16.16 X 10² pmol/ 24 h/ pair CA); the release rate of juvenile hormone acid II (JHA II) was constantly low from any age group. Male CA from different time intervals of a 24 h photoperiod showed different abilities to release JHAs.

05-069

EFFECT OF DIMBOA ON NUTRITIONAL INDICES, DIGESTIVE PROTEASES AND DETOXIFICATION ENZYMES OF *SESAMIA NONAGRIOIDES* (LEPIDOPTERA: NOCTUIDAE) LARVAE

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The hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) was assessed for its effect on the digestive physiology of larvae of the stalk corn borer *Sesamia nonagrioides* Lef. Nutritional indices and enzymatic activities were determined for larvae feeding on a DIMBOA-containing diet for the first two days of the third instar (short-term assays), for the full third instar (middle-term assays), and from neonates to third instar (long-term assays). In long-term feeding assays, DIMBOA reduced the relative growth rate (RGR), and the efficiency of conversion of ingested food (ECI), without affecting the relative consumption rate (RCR). However, DIMBOA had no effect on RGR, RCR, and ECI in short- and middle-term feeding assays .

Trypsin, chymotrypsin, elastase, carboxypeptidase A and B, and microsomal oxidase activities were significantly increased by DIMBOA in middle term-assays, whereas esterase activity was reduced. Elastase in short-term assays and chymotrypsin, elastase and microsomal oxidase activities in long-term assays were also increased. Moreover, DIMBOA inhibited *in vitro* the activities of both carboxypeptidases, aminopeptidase, glutathione S-transferase and esterase, but it had no effect on the endoproteases.

The implications of the altered levels of proteases and detoxification enzyme activities on the digestive physiology of larvae feeding on DIMBOA-containing diets are discussed.

05-071

HEAT SHOCK COGNATE PROTEINS AND DEGRADATION OF JUVENILE HORMONE ESTERASE IN *MANDUCA SEXTA* (LEPIDOPTERA: SPHINGIDAE).

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A heat shock cognate protein (Hsc) is involved in degradation of hemolymph proteins such as juvenile hormone esterase (JHE), in the pericardial cells of the tobacco hornworm, *Manduca sexta*. Efficient removal of JHE is effected by receptor-mediated endocytosis by the pericardial cell complex and is essential for larval development. Endocytic vesicles fuse with the *trans*-Golgi network where proteins are sorted for return to the cell surface, or for degradation in lysosomes. Immunohistochemical studies suggest that the Hsc is in the *trans*-Golgi network or the prelysosomal compartment of the pericardial cells. This Hsc appears to be unique in size and immunological cross-reactivity relative to other heat shock protein families. Immunoprecipitation experiments indicate that the binding of Hsc to JHE is reduced by mutagenesis of specific residues in the putative lysosome-targeting sequence of JHE. We hypothesize that the Hsc acts as a chaperone of JHE from the *trans*-Golgi network into the prelysosomal compartment for transfer to lysosomes for degradation. This is the first report of Hsc involvement in degradation of an extracellular protein.

05-072

EXTRACTION AND CHARACTERIZATION OF THE DARK COLOR INDUCING NEUROHORMONE FROM THE CORPORA CARDIACA OF *LOCUSTA MIGRATORIA*.Y. Yerushalmi¹, S. Tanaka², Y. Milner³, M.P. Pener¹.

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We extracted and purified a dark color-inducing neurohormone (DCIN) from the corpora cardiaca (CC) of a normal strain of *Locusta migratoria* by employing a novel bioassay based on an albino strain of this species in which the DCIN is absent or physiologically non-functional. Dried methanol extracts of the CC of the normal strain, resuspended and injected in olive oil, induced dose-dependent darkening of the albino hoppers. Injections in aqueous media were ineffective. Methanol extracts of normal CC, submitted to gel filtration on Sephadex LH-20 column, followed by reverse phase HPLC with acetonitrile gradient, yielded a single active fraction. Mass spectrogram of this active fraction showed a single peak of 3,752 dalton. Preliminary sequence analysis revealed the first 16 amino acids of the DCIN. The latter result, and also those of some further experiments, showed that the DCIN is distinctly different from any other known dark color promoting insect neurohormone, including the MRCH (=PBAN).

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05-074

SOURCE OF THE LIPIDS IN MOSQUITO OOCYTES.

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Mosquito eggs, like all animal eggs, contain large amounts of lipids. The lipids are needed to form membranes and to supply energy to the developing embryo.

Mature oocytes of the mosquito, *Aedes aegypti*, contain on a weight basis about 30% lipids. The lipids could be synthesized in the ovary, or they could be transported to the oocyte from fat body or from the gut after a blood meal. Experiments with radiolabeled water demonstrated that ovaries incorporate a small amount of label into lipids, mostly into phospholipids and triacylglycerol. More than 90% of this label is found in glycerol and less than 10% is incorporated into fatty acids, indicating that lipids from other sources are transported to the oocytes and taken up.

Insects transport lipids by lipophorin, their major hemolymph lipoprotein. Radiolabeled fatty acids presented by lipophorin as acylglycerol or as free fatty acids are readily taken up by mosquito ovaries in vitro.

Acylglycerols can only be taken up if they are first hydrolyzed by a lipase to form free fatty acids and glycerol. In homogenates of ovaries lipase activity was found. The subcellular localization of the lipase will be examined.

This work was supported by grant GM 29238 from the National Institute of Health

05-073

CRYO-ELECTRON MICROSCOPY OF A HIGH DENSITY LIPOPHORIN FROM THE HEMOLYMPH OF THE HAWKMOTH *MANDUCA SEXTA*Rik van Antwerpen¹ and John C. Gilkey².

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In the hemolymph of the hawkmoth *Manduca sexta*, lipids are transported by a high density lipoprotein, called high density lipophorin (HDLp). We have analyzed the structure of this lipoprotein, using cryo-electron microscopy and computer-assisted image processing. HDLp was studied in a frozen-hydrated condition, without chemical fixation or any form of staining. As the contrast of cryo-electron micrographs is directly related to the distribution of mass density within the actual preparation, distinct high density structures in our micrographs are indicated to represent apoprotein domains within the lipoprotein particle. Different projections of HDLp with three or four separate, globular domains of high density were recognized, which suggests that the observed domains may be organized at tetrahedral positions relative to each other. This organization is very different from the organization of high density in cryo-electron micrographs of the human low density serum lipoprotein (LDL). The present results demonstrate that cryo-electron microscopy may visualize structural properties of lipoprotein particles that, to date, can not be detected by any other means.

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05-075

ENZYMATIC ANALYSES OF URICOTELY IN THE MOSQUITO *Aedes Aegypti* (DIPTERA: CULICIDAE)P. von Dungen & H. Briegel
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When feeding on blood, female mosquitoes ingest protein in excess of the requirements for oogenesis. The surplus of nitrogen is removed from the metabolism by the synthesis of uric acid which is catalized by xanthin dehydrogenase (XDH). XDH activity profiles were established for sugarfed and for bloodfed females of *Aedes aegypti*. After discharge of the meconium, XDH activity gradually diminished in females maintained on sucrose. After bloodmeal however, XDH activity increased substantially, and it returned to pre-bloodmeal levels upon termination of digestion.

In females given measured volumes of blood by enema, XDH activity was proportional to the protein input and urate excretion as well. The primary site of XDH activity is the fatbody. The activity profiles have also been explored in ageing females and in comparison to the proteolytic enzymes within the midgut after blood meal.

Considerable XDH activities were observed in fourth instar larvae and teneral pupae as well. This result was surprising because in water-living instars or insect species uricotelous has not been recorded before. The possibility of storage excretion of urate in some form by the aquatic mosquito larvae remains to be investigated.

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05-076

PROLINE IS A WIDESPREAD FUEL FOR FLIGHT IN BEETLES

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Flying insects utilize various substrates to power the flight muscles: carbohydrates are known to be the predominant or exclusive fuel for flies and bees, whereas locusts and butterflies oxidize lipids during long-distance flights. The tsetse fly, but also the Colorado potato beetle are well-known examples of gaining energy for flight by partially oxidizing the amino acid proline. But is this phenomenon restricted to a few special cases or is this type of energy metabolism much more ubiquitous than previously thought?

Detailed analysis of maximal activities of key enzymes of energy metabolism in the flight muscles, of oxidation of various substrates by isolated flight muscle mitochondria and of changes of concentration of metabolites during flight in haemolymph and flight muscles have shown that proline is an important fuel for flight in scarabaeid (*Pachnoda sinuata*, *Onitis pecuarius*, *Scarabaeus deludens*, *Melolontha melolontha*, *Trichostetha albopicta*, *Trichostetha fascicularis*) and meloid beetles (*DeCAPotoma lunata*). However, no proline was detected in the haemolymph of buprestid beetles.

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05-077

HAEMOLYMPH SUGARS AND THE CONTROL OF THE PROVENTRICULUS IN THE HONEY BEE *APIS MELLIFERA*

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In insects feeding on liquid food, both haemolymph osmolarity as well as titres of specific nutrients have been observed to play a role in modulating crop-emptying rates. In honey bees, haemolymph osmolarity has been suggested as the main factor affecting the flow of sugar solution from the social crop to the gut via the proventriculus. We present evidence indicating that the level of trehalose in the haemolymph controls crop-emptying rates through the proventriculus in the honey bee. Bees were trained to collect a given amount of sugar solution of different concentration. After voluntary feeding, they were caged for different periods and then dissected, in order to determine crop content, rectal pouch content and haemolymph sugars. In order to distinguish between haemolymph osmolarity and nutrient titres as factors controlling the activity of the proventriculus, groups of trained foragers were injected with nutritive carbohydrates such as trehalose and glucose, and control groups with non-nutritive carbohydrates (fucose, sorbose), which produce a similar increase in haemolymph osmolarity but cannot be metabolised by bees. Measurements of crop-emptying rates, urine production, metabolic expenditure and sugar levels in the haemolymph indicated that the sugar flow through the proventriculus rather than the flow of solution is controlled, and that an increase in trehalose levels in the haemolymph, irrespective of the concomitant increase in osmolarity, directly inhibited the activity of the proventriculus.

05-078

LIPID METABOLISM IN *Rhodnius prolixus* MIDGUT (HEMIPTERA)

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In *Rhodnius prolixus* during the period of oogenesis the lipophorin transfers phospholipids to the developing oocytes and is reloaded with more phospholipids at the fat body or at the midgut. The interplay among this tissues depend on the physiological state of insect. During the period of intensive dietary absorption lipophorin takes up phospholipids preferentially at the midgut and later on, when blood digestion slow down, at the fat body. In this work we studied incorporation of free fatty acids by midgut of *Rhodnius*. When radioactive C¹⁴-Oleic acid was injected into hemocel of females the radioactivity was recovered associated with lipophorin. Ten minutes after injection the radioactivity disappeared from hemolymph, and was recovered mainly at the midgut. The capacity of midgut to incorporate C¹⁴-Oleic acid increased until the tenth day after feeding. However, the midgut ability to transfer lipids to lipophorin, decreases from day one onward. The C¹⁴-Oleic acid incorporated by the midgut was distributed among phospholipids (60%) and neutral lipids (40%). The fate of C¹⁴-lipids synthesized by the midgut was followed and it was observed that diacylglycerol is the major lipid released to lipophorin particles. While the C¹⁴-phospholipids synthesized by the midgut are not transferred to lipophorin but it is probably used for membrane synthesis. C¹⁴-Phospholipids were found associated with the luminal content of *Rhodnius*.

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05-080

PURIFICATION AND PARTIAL CHARACTERIZATION OF A LUTEIN-BINDING PROTEIN FROM THE MIDGUT OF THE SILKWORM *Bombyx mori*

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A carotenoid-binding protein was purified from fifth instar larval midgut of *Bombyx mori* by a combination of ammonium sulfate fractionation and three chromatographic procedures: gel filtration, chromatofocusing, and anion exchange chromatography. The protein has a pI of 5.4 and an apparent molecular mass of 35,000 Da, as determined by a linear gradient SDS PAGE. The carotenoid-protein complex is water soluble and more stable than the carotenoid or protein alone. The carotenoid moiety was identified by thin layer chromatography, light absorption spectroscopy, and HPLC as *all-trans*-lutein. Lutein is specifically and stoichiometrically bound to the protein, with a ratio of three mols of carotenoid per mol of protein. Binding of lutein (absorption maximum in hexane at 454 nm) to the apoprotein results in a marked red spectral shift of about 38 nm, giving rise to absorption maxima at 432 nm, 462 nm and 492 nm in 20 mM Tris-HCl, pH 7.0. The carotenoid-protein complex is characterized by fine spectral structure indicating that lutein is in a relatively rigid environment. This protein is distributed in equal amounts throughout the midgut and in all developmental stages of the larval *B. mori*. Supported by NIH grant 50008.

05-079

IN VIVO PHYSIOLOGICAL STUDIES OF LIPID TRANSFER PARTICLE IN *BOMBYX MORI* USING ANTIBODIES

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Lipid transfer particle (LTP) is a high molecular weight complex of three apoproteins and lipids. *In vitro* studies showed that LTP transfers lipids between lipophorins, from fat body to lipophorin and from lipophorin to the developing oocyte, but the physiological role of LTP in lipid transfer reactions in insect hemolymph is not yet understood. I have studied the functional properties of *Bombyx mori* LTP *in vivo*, using antibodies.

LTP and lipophorin concentrations in hemolymph from 4th instar larvae to adult were determined by immunodiffusion. The total amount of LTP in hemolymph was rapidly increased from 5th instar day 0 to day 4, then dropped gradually at pupation. The protein concentration ratio between LTP and lipophorin was 1:15, all through the 4th and 5th instar larval stages, and 1:25 during the pupal stage. To clarify the physiological role of LTP lipid transfer activity *in vivo*, anti LTP IgG was injected in larvae and pharate adults. In larvae injected with anti-LTP IgG, low density lipophorin (LDLp) was diminished, and only high density lipophorin (HDLp) existed in the hemolymph. In addition LTP was shifted to a higher density fraction than the control. In adults injected with anti-LTP IgG, all lipophorin was observed as LDLp, HDLp was completely diminished or was not produced. On the other hand, control adults injected with preimmune rabbit IgG had two type of different density lipophorin. These results indicated that anti-LTP IgG can block the lipid transfer activity of LTP in hemolymph and suggest that the most important role of LTP is not to transfer lipids between lipophorin particles.

05-081

THE ROLE OF FATTY ACIDS PRODUCED IN SCALE-COVER AND SECRETORY MATERIALS OF FIVE SCALE INSECTS (COCCOIDEA:HOMOPTERA)

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The quantity and quality of the fatty acids produced in their scale-covers and secretory materials were investigated using GLC experiments.

The experiments aimed to find out any relationship may differentiate among three diaspidid scales representing the three tribes of family Diaspididae (long scale-cover, circular scale-cover and those puparium scale-cover), as well as two soft-scale insects, one belonging to family Coccidae and the other belonging to the family Margarodidae.

The puparium, *Parlatoria zizyphus* (Lau.) was the richest producer of palmitic (24%), oleic (20%) and palmitoleic (17%). The long-scale, *Lepidosaphes beckii* (Newm.) was the richest producer of arachidic (15.8%) and linolenic (10.5%). The circular, *Aonidiella aurantii* (Mask.) was the richest in myristic (33.4%) followed by palmitic (20%).

The coccid, *Ceroplastes rusci* (L.), however, surpassed all the examined insects in myristic (49%) and lauric (12%), but the margarodid surpassed in the production of capric (39.5%).

05-082

ISOFORMS OF *BOMBYX MORI* ECDYSONE RECEPTOR AND THEIR EXPRESSION DURING METAMORPHOSIS

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Using 5'-RACE methods, two different 5' ends of *Bombyx mori* ecdysone receptor homologue (BmEcR) cDNA were obtained. They were different in the A/B region, but same in the downstream of C (DNA binding) region. As BmEcR was indicated to be a single copy gene (Swevers *et al.*, 1995), it is suggested that they are generated by different promoter usage or alternative splicing, like isoforms of *Drosophila melanogaster* EcR (DmEcR). One of these cDNAs was homologous to DmEcR A isoform and another was homologous to B1 isoform, so they were named BmEcR-A and BmEcR-B1, respectively. Both of BmEcR-A and BmEcR-B1 specific probes detected single 6 kb message in the northern analysis, indicating that those mRNAs are about in a same size. Both isoforms were expressed in tissue-specifically at the larval-pupal metamorphosis. During the last larval instar, expression of BmEcR in the wing discs was maximal on the day of wandering, indicating that the expression of BmEcR is prerequisite for the wing discs to respond to the drastic increase of hemolymph ecdysteroid titer.

05-084

ECDYSONE AND 20-HYDROXYECDYSONE AFFECT THE SENSITIVITY OF EPIDERMIS TO ECDYSTEROIDS DIFFERENTLY IN THE SILKWORM, *BOMBYX MORI*.

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In order to explore the different effect of ecdysone and 20-hydroxyecdysone on larval development in the silkworm, *Bombyx mori*, larval epidermis were cultured in Grace's medium and their responses to ecdysteroids were investigated. The epidermis dissected from newly-ecdysed fourth instar larvae responded to 20-hydroxyecdysone ≥ 10 ng/ml by forming new cuticle (apolysis) within 3 days, and the epidermis dissected from the fifth-instar larvae induced by ecdysone responded to almost the same level of 20-hydroxyecdysone *in vitro*. On the other hand, even 50 ng/ml of 20-hydroxyecdysone did not induce apolysis in the epidermis of the larvae induced by 20-hydroxyecdysone. These results suggest that the two ecdysteroids affect the sensitivity of epidermis to ecdysteroids differently and that the fifth-instar larvae induced by 20-hydroxyecdysone died without apolysis because of the decline in the sensitivity of epidermis to ecdysteroids.

05-083

THE ECDYSONE REGULATED PUFFS IN *CERATITIS CAPITATA* SALIVARY GLANDS

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The steroid hormone ecdysone acts as a temporal signal to coordinate tissue specific morphogenetic changes in insects through the activation of a large number of primary and secondary genes. Extensive studies in *Drosophila* have answered several questions concerning the mechanisms of ecdysone response as well as have raised new ones. Given the wide array of ecdysteroids among insects and the differences in their life cycles, parallel studies in other insect species and particularly in those of economic importance, may reveal unique future of hormone regulation, thus establishing the basis for developing species-specific pest control methods. We have undertaken such studies in the Medfly and our data from the cytogenetic analysis of the ecdysone action are presented in this report.

Sequential changes in the puffing activity of *C. capitata* salivary gland chromosomes take place during the late larval and prepupal stages. The most remarkable changes were observed at the jumping stage during which the titer of ecdysone increases in the haemolymph and larvae jump out of the medium in order to pupate. Tissue culture experiments in the presence of ecdysone and inhibitors of protein synthesis, indicated that most of the changes in the puffing activity were due to the ecdysone. A small number of puffs are either suppressed or induced directly by ecdysone while the majority of the ecdysone depended puffs are induced indirectly and required protein synthesis in addition to the hormone.

05-085

INTEGUMENT CHARACTERISTIC TO THE LAST LARVAL STADIUM IN THE SWEET POTATO HORNWORM, *AGRIUS CONVULVULI* (LEPIDOPTERA; SPHINGIDAE)

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The sweet potato hornworm, *Agrius convulvuli*, pupates after the 5th or the 6th larval stadium. Most of larvae became the 6th stadium by malnourishment after the 3rd stadium. There were many processes on entire surface of the cuticle at immature stadia. The processes disappeared at the last larval stadium. The integument of the 5th stadium larvae was similar to that of the 4th stadium day 2 larvae in its inner morphology. When the integument of the 4th stadium day 1 larvae was cultured in the MGM-450 medium containing 20-hydroxyecdysone, apolysis was induced, but new integument was not formed. However, from the morphological changes of epidermis it was assumed that the change to the last stadium type integument was initiated before day 1 of the penultimate stadium. Cuticular proteins were analysed by native PAGE, and one of cuticular proteins existed up to the 4th stadium proved to disappear at the last stadium.

05-086

STUDIES ON THE MOLECULAR STRUCTURE OF JUVENILE HORMONE AND ITS BIOSYNTHETIC PATHWAY IN THE HOUSEFLY *MUSCA DOMESTICA*.

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The housefly, *Musca domestica* is a nuisance pest in Japan and occurs as both anautogenous and autogenous strains. Egg development in the females is known to be under control of ecdysteroids and is affected by synthetic juvenoids. Our studies investigated the biosynthetic pathway of the juvenile hormone production and its role in oögenesis in the female housefly using the *in vitro* radiochemical assay for juvenile hormone biosynthesis.

Ring glands incubated with [³H]-methyl methionine biosynthesized radiolabelled product that co-chromatographed on TLC with synthetic juvenile hormone bisepoxide (JHB₃) and occurred at a constant rate over a 6 hr incubation period. Since rates of egg development varied between individual female flies, rates of oögenesis were standardized by staging according to the technique of Adams, 1974. Rates of JHB₃ biosynthesis by female ring glands in strains of autogenous (Misaki strain) and anautogenous (Chubo strain) were different during the first gonotrophic cycle. The autogenous strain showed a rapid increase in biosynthesis during the early stage of oögenesis (3-4) followed by a plateau (stage 5-6). A decrease in the rate was seen at stage 7, but was followed by an increase (stage 8-9) to a level above that seen during the previous stage (5-6). The rates of biosynthesis of ring glands from females with mature eggs (stage 10) decreased to zero level. In contrast, the anautogenous strain did not show any further increase during the late vitellogenic stage (8-10).

05-087

PURIFICATION AND CHARACTERIZATION OF JUVENILE HORMONE BINDING PROTEIN FROM CYTOSOL OF THE SILK GLAND OF THE SILKWORM, *BOMBYX MORI*

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A juvenile hormone binding protein (JHBP) has been isolated from cytosol of silk glands of *Bombyx mori* by ion-exchange chromatography, gel filtration, chromatofocusing and polyacrylamide gel electrophoresis (PAGE). The cytosol was provided by ultrasonic treatment of the silk glands in a 50 mM Tris-HCl buffer containing protease inhibitors, 7.4 pH. The JHBP has a relative molecular mass of 29 kDa, determined by sodium dodecyl sulfate PAGE. Chromatofocusing analysis indicated that the JHBP is an acidic protein with pI 5.8. The protein exhibits a dissociation constant of 1.2×10^{-8} M for Juvenile hormone III.

05-088

JUVENILE HORMONE MEDIATED NEUROENDOCRINE CONTROL OF SEXUAL BEHAVIOUR IN MALE *BLATTELLA GERMANICA* LINN. (BLATTOIDEA ; BLATTELLIDAE)S. C. Pathak¹, Rita Bhandari²

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The effects of various components of the neuroendocrine system on the sexual behaviour of male cockroach *Blattella germanica* Linn. were investigated with the help of frontal ganglionectomy, cauterisation of the cerebral neurosecretory cells, chemical inhibition of the corpus allatum and administration of exogenous Juvenile hormone. The results reveal that the sexual behaviour is completely blocked when any of the three components viz. frontal ganglion, cerebral neurosecretory cells or the corpus allatum is removed / inhibited. Administration of exogenous Juvenile hormone brings about resumption of this behaviour, after about 24 hours of such administration.

05-089

IN VIVO* AND *IN VITRO* REGULATION OF THE CORPUS ALLATUM BY BRAIN IN THE HEMIPTERAN *PYRRHOCORIS APTERUSM. Hodkova, T. Okuda¹

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Surgical interventions to the neuroendocrine system *in situ* indicate that photoperiodic regulation of reproduction in *Pyrrhocoris apterus* is mediated by pars intercerebralis (PI) of the brain. The complexes of brain-suboesophageal ganglion-corpora cardiaca-corpora allatum extirpated from short day (diapause) and long day (non-diapause) females were subjected to extirpation of the PI or transection of nerves from brain to the corpus allatum (CA). Thereafter, the activity of operated and control complexes was measured either by ovarian maturation *in vivo* (after transplantation to diapause or allatectomized females) or by synthetic activity of the CA *in vitro*. It was found that the activity of the CA was inhibited from the PI in both short day and long day complexes but the inhibition induced by short days was stronger. The inhibitory factor acted via both nervous and humoral pathways.

05-090

ALLATOSTATIN CONTAINING GRANULES IN HEMOCYTES OF THE COCKROACH, *DIPLOPTERA PUNCTATA* (Blattaria, Blaberidac)

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Among the hemocytes in the hemolymph of *Diploptera punctata* is a population with granules that immunoreact with antibodies against a *D. punctata* allatostatin (A-P-S-G-A-Q-R-L-Y-G-F-G-L-NH₂). The allatostatins isolated from this cockroach are a family of neuropeptides that, among other functions, inhibit juvenile hormone synthesis in the cockroach and related insects, e.g., cricket. Allatostatin positive hemocytes vary from 6 to 13 per cent of the hemocytes. Older animals (70 days after adult emergence) have fewer (~2%) than those within a week of adult emergence or in the nymphal stage. These hemocytes exhibit a fine structure consistent with the production and storage of allatostatin immunopositive material. The potential contribution of hemocytes to the regulation of the juvenile hormone synthesis by the corpora allata will be discussed.

05-092

Concentration of cyclic AMP and Activity of cyclic AMP-dependent Protein Kinase in Chestnut Weevil, *Curculio dentipes*

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The concentration of cyclic AMP (cAMP) of whole body and activity of cyclic AMP-dependent protein kinase (PKA) in partial purification were measured in chestnut weevil, *Curculio dentipes* according to the non-overwintering, wintering, and overwintering larvae. cAMP concentration and PKA activity measured by HPLC and Liquid Scintillation Counter, and investigated the correlation of cAMP and PKA

The cAMP concentration were found to be the maximum at the non-overwintering larvae (0.57 μ M/g) and decreased at the wintering larvae (0.14 μ M/g). At the overwintering larvae, it was increased again (0.29 μ M/g). Also, the activity of PKA were showed the highest level at the non-overwintering larvae (2.56 unit/mg) and decreased at the wintering larvae (0.62 unit /mg) in calcium phosphate gel purification step, whereas it increased again at overwintering larvae (2.07 unit/mg).

Therefore, cAMP concentration and PKA activity were shown to be similar aspect. This results were suggested that the accumulation of fat body in non-overwintering larvae appeared to be more closely related to largest ingestion prior to diapause and that cAMP concentration and PKA activity increased with prepare of larval-pupal ecdysis and termination of diapause in overwintering larvae, whereas cAMP and PKA level decreased with fat body degradation at wintering larvae for use of energy source.

05-091

INTRACELLULAR SIGNAL TRANSDUCTION OF PBAN IN THE SILKWORM, *BOMBYX MORI*.

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Pheromone biosynthesis activating neuropeptide (PBAN) regulates sex pheromone production in a number of lepidopteran species. In *B. mori*, PBAN regulates the step of the fatty acyl reduction, the final step of the bombykol biosynthetic pathway to produce the terminal hydroxyl group. In order to probe the biochemical steps as well as underlying mechanisms regulated by PBAN, we have tested the effects of pharmacological agents on bombykol production by using an in vitro assay with pheromone glands. In addition, we have detected acyl CoA reductase activity in the pheromone gland of *B. mori* by using palmitoyl CoA as a substrate. The acyl CoA reductase requires NADPH, but not NADH, as a proton donor. Cell-free production of bombykol was attained by incubating the pheromone gland homogenate in the presence of NADPH, ATP and CoA. Removal of either NADPH, ATP or CoA from the incubation medium resulted in no stimulation of bombykol production, suggesting that the final two steps of the bombykol biosynthetic pathway are conducted by acyl CoA synthetase and acyl CoA reductase, sequentially. Based on these results, we discuss the biochemical mechanisms of sex pheromone production regulated by PBAN in *B. mori*.

05-093

TERMINATION OF SEX PHEROMONE PRODUCTION IN MATED FEMALES OF THE SILKWORM MOTH, *BOMBYX MORI* L.

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A mating duration of more than 6 h was necessary to permanently terminate the production of the sex pheromone (bombykol) in the silkworm moth, *Bombyx mori* L. (Lepidoptera: Bombycidae), although the female formed a bursa copulatrix including a spermatophore and laid fertilized eggs even after mating for only 0.5 h. The 6-h mated female again produced bombykol if given an injection of synthetic pheromonotropic neuropeptide (PBAN), which is known to activate pheromone biosynthesis in a virgin female. Extracts of brain-suboesophageal ganglion (SG) complexes, which were removed from 6-h and 24-h mated females, showed strong pheromonotropic activities. These results indicated that the pheromone gland of the mated female maintained its ability to biosynthesize bombykol; however, it could not produce pheromone due to a suppression of PBAN secretion from the SG. Furthermore, bombykol titers did not decrease after mating in females with a transected ventral nerve cord, even after the injection of a spermatophore extract, suggesting that the suppression of PBAN secretion was mediated by a neural signal and not by a substance in the spermatophore. The mated females accumulated (10E,12Z)-10,12-hexadecadienoic acid, a precursor of bombykol biosynthesis, in their pheromone glands as did decapitated females.

05-094

PURIFICATION, CHARACTERIZATION AND PARTIAL AMINO ACID SEQUENCE OF INITIATORIN, A PROSTATIC ENDOPEPTIDASE OF THE SILKMOTH, *Bombyx mori* (L.) (Lepidoptera: Bombycidae)

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Initiatorin is a serine-endopeptidase secreted from the glandula prostatica gland of the silkworm, *Bombyx mori*. It acts as an initiator for sperm maturation in the spermatophore: (1) on inducing for motility of apyrene spermatozoa, (2) on dissociating eupyrene sperm bundles, (3) on initiating the arginine degradation cascade by degradation of arginine-rich proteins, and (4) on activating the zymogen of arginine carboxypeptidase which produces free arginine from peptide.

Purification of initiatorin was monitored by assay of hydrolyzing activity of BAEE (N α -benzoyl-L-arginine ethyl ester). Using affinity-chromatography with *p*-aminobenzamidine (ABA) as a ligand which is the strong inhibitor of initiatorin (82% inhibition by 0.1mM ABA), this enzyme was purified over 200-fold from the glandula prostatica of males.

Against BAEE as a substrate, Km-value of this enzyme is 2.43×10^{-4} M. Molecular weight of the purified initiatorin was determined as 30KDa by SDS-PAGE.

The determined N-terminal amino acid sequence of this initiatorin preparation is the same as that of another initiatorin preparation purified from the spermatophore of which molecular weight is 29KDa (Aigaki et al., 1994).

05-096

PROTEASES IN THE TESTIS OF THE SILKMOTH, *BOMBYX MORI* (L.) (LEPIDOPTERA: BOMBYCIDAE)

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In the testis of the silkworm, *Bombyx mori* bundle of anucleate apyrene sperm dissociate and separate apyrene sperm move. On the other hand, it was found that initiatorin, a serine-endopeptidase localized in the glandula prostatica has capacities not only to dissociate bundles of eupyrene sperm but also to induce motility of apyrene sperm in the spermatophore. These findings suggest that also the dissociation of bundles of apyrene sperm and their motility acquisition in the testis may be caused by a protease(s).

Two proteases were detected in the testis during development of the silkworm. One shows L-alanine-*p*-nitroanilide (ANA)-hydrolyzing activity and the other N α -benzoyl-L-arginine ethyl ester (BAEE)-hydrolysing activity. These two enzymes were able to be extracted from the testis, only when the extracting solution contains a high concentration of cetyltrimethylammonium bromide (CTB). The enzyme activity of the former aminopeptidase (ANAAse) increases from day 3 of the 4th instar larva, reaches a maximum at day 2 of the 5th instar larva and then decreases. The enzyme activity of the latter endopeptidase (BAEEase) showed similar developmental changes to ANAAse. These activity maxima correspond to the time when a large number of spermatoocytes of apyrene sperm appear.

05-095

INITIATORIN INHIBITOR OF THE MALE GLANDULA PROSTATICA OF THE SILKMOTH, *BOMBYX MORI* (L.) (Lepidoptera : Bombycidae)

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Initiatorin is a serine-endopeptidase secreted from the glandula (g.) prostatica of the male silkworm, *Bombyx mori*. Like trypsin, this enzyme has a capacity to hydrolyze N α -benzoyl-L-arginine ethyl ester (BAEE). Initiatorin was first precipitated ammonium sulfate 30-80% at saturation, then purified using FPLC on an affinity-chromatography fixed with *p*-aminobenzamidine as a ligand. At that time when the extract from the g. prostatica was treated with ammonium sulfate, BAEEase activity was highly increased, suggesting that the presence of an inhibitor in this gland. Thus, this initiatorin inhibitor (PII) was purified from the g. prostatica. Gel filtration FPLC was carried out on a Superdex™75. Prepared PII is a heat-stable peptide of which molecular weight is under 10kDa. This natural inhibitor inhibits BAEEase activity of bovine trypsin as well as that of initiatorin. The inhibition on trypsin is stoichiometric and considered to be noncompetitive. The dissociation constant was calculated as about 1.0mg per ml.

Its physiological functions may be not only to protect cells from harmful effect of initiatorin but also to control motility acquisition of apyrene sperm.

05-097

THE ANGIOTENSIN CONVERTING ENZYME RELATED CARBOXYDIPEPTIDASE OF THE BUFFALO FLY EXPRESSED IN MATURING TESTES.

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The angiotensin converting enzymes (ACEs) are membrane bound carboxydiptidases ubiquitous in mammalian systems. They are involved in the regulation of the activities of several bioactive peptides. An enzyme similar to the ACEs has been purified and cloned from the adult stage of the buffalo fly (*Haematobia irritans exigua*) - a close relative of the horn fly. The buffalo fly enzyme, HieACE, is not membrane bound and behaves as a 70kD doublet in reducing SDS-PAGE.

HieACE was purified from whole fly preparations by lectin and ion exchange chromatographies. The purified HieACE has similar enzymatic activity to the mammalian ACEs and displays similar specificity to ACE peptide substrates such as angiotensin I, bradykinin, substance P and cholecystokinin. Sequences of peptides resulting from an endo-Lys-C digest of purified HieACE were used to design PCR primers and a DNA fragment amplified from adult buffalo fly cDNA was used to screen a cDNA library. The coding region of a full length cDNA clone predicted an ACE-like carboxydiptidase with 42% amino acid identity to mammalian testicular ACEs.

An antiserum raised to the purified HieACE has shown that in the unfed adult fly, HieACE expression was restricted to the compound ganglion and posterior midgut. However, in a curious parallel to the mammalian system, expression of the enzyme is induced in the maturing male reproductive system after the first blood meal.

05-098

EXPRESSION OF ANGIOTENSIN CONVERTING ENZYME HOMOLOGUES IN THE LARVAE OF HIGHER DIPTERA.

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The characterisation and cloning of the adult buffalo fly homologue of the mammalian angiotensin converting enzyme (ACE) has been recently described (Wijffels *et al.* 1996). The fly homologue, HieACE, has enzymatic similarities to the ACEs and exhibits 42% sequence identity with testicular ACE. HieACE expression in the unfed adult fly is restricted to the compound ganglion and posterior midgut, although expression is induced in the male reproductive tissues after the first blood meal.

However, expression of HieACE and its homologues in other diptera is not restricted to the adult stage. The ACE-like enzymes were detected by Western blot analyses in the third instar stages of the buffalo fly, sheep blowfly (*Lucilia cuprina*) and *Drosophila*, and fourth instar larvae of the mosquito, *Aedes vigilax*. In fact, all larval stages of the sheep blowfly expressed the *Lucilia* ACE homologue. In these species, HieACE and its counterparts were approximately 70kD in size and not membrane bound. The ACE-like enzymes have a broad tissue distribution in the third instar larvae of the buffalo fly and sheep blowfly. HieACE and its *Lucila* homologue were detected throughout the gut, the salivary glands and the compound ganglion. This contrasts with the restricted tissue distribution of the adult flies of these species and suggests a different role for the dipteran ACE-like enzymes in the larval stages.

Wijffels, G., Fitzgerald, C., Gough, J., Riding, G., Elvin, C., Kemp, D., and Willadsen, P. (1996) "Angiotensin Converting Enzyme" of the Dipteran Species, *Haematobia irritans exigua*, and its Expression in Maturing Male Reproductive System. *European J. Biochem.* (in press)

05-100

PURIFICATION AND CHARACTERIZATION OF THREE ISOFORMS OF VITELLIN IN *Rhodnius prolixus*

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Vitellogenins (VTG) are high molecular weight phospholipoglycoproteins. They are taken up from hemolymph by the oocytes by receptor-mediated endocytosis. After incorporation by the ovaries, they are called vitellins (VT). Vitellin, the main protein of *Rhodnius prolixus* oocytes was purified by means of ion exchange chromatography DEAE TOYO-PEARL. Three VT isoforms were identified and they were called VT₁, VT₂, VT₃.

All VT isoforms have the same neutral lipids, but were heterogenous with respect to phospholipids composition. VT₁ have only phosphatidylcholine, whereas VT₂ showed phosphatidylcholine, cardiolipin, and a non-identified lipid and VT₃ had phosphatidylcholine, phosphatidic acid, sphingomyelin, cardiolipin, and the same non-identified lipid. The phosphate content of each isoform were analyzed after lipid extraction and it was also heterogenous, VT₁ being the less phosphorylated and VT₃ the most heavily phosphorylated isoform. Sugar composition was analyzed by means of paper chromatography. VT₁ have mostly glycose than mannose and in contrast VT₃ have mostly mannose than glycose.

When metabolic labelled ³²Pi-VT isoforms were injected into the hemocel of vitellogenic females, VT₃ was taken up with a rate about three times higher than that observed for VT₁ and VT₂.

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05-099

DISTRIBUTION AND ACCUMULATION OF STORAGE PROTEIN IN OVARY OF *HYPHANTRIA CUNEA* DRURY

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The accumulation and uptake of storage protein in ovary of fall webworm, *Hyphantria cunea* Drury, were investigated using autoradiograph, Western blot, and immunocytochemistry. Storage protein is found at high level in hemolymph as well as fat body during pupal stage, while in ovary storage protein begin to appear at trace level in 6 day-old pupae and accumulate until adult stage.

During pupal stage storage protein in hemolymph penetrates through the layer of tunica propria and gains access to oocyte. Storage protein uptake occurs across the brush border from the follicular epithelial cells to the oocyte, producing rapid accumulation of storage protein in yolk spheres.

In the oocyte, storage protein is incorporated to in the small protein granules, which are fused together to form large yolk sphere. This transportation of storage protein seems to continue until the formation of vitelline membrane.

05-101

A Highly Phosphorylated Low Molecular Weight Protein from the Oocytes and Hemolymph of *Rhodnius prolixus* : Isolation and Partial Characterization.

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Vitellin is a phospholipoglycoprotein which composes the yolk of oviparous organisms. When *Rhodnius* females are fed with blood enriched with ³²Pi it is possible to label its phosphoproteins metabolically. This approach showed that besides Vitellin a second phosphoprotein is also present in these oocytes. We have isolated this protein through gel filtration on Sephadex G-75, anion exchange on DEAE Toyo Pearl and Mono Q 5/5 HR. The purified molecule is a single band on Tricine gels with 8 kDa of molecular weight but it shows a native weight of 17 kDa. Aminoacid composition shows a high content of Serines (25 %) and these residues are the only targets for phosphorylation. NH₂- terminal sequencing shows no significant homology with available sequences. Another phosphoprotein was also isolated from hemolymph. Its identity with the oocyte phosphoprotein was confirmed by NH₂- terminal sequencing. Both radiolabelled molecules are ready taken up by growing ovaries following injection into hemocel of adult females. The phosphoprotein present inside the oocyte is a substrate for purified Casein Kinase II, which indicates the possibility of post-endocytotic processing. During embryogenesis, a decrease in this phosphoprotein is detected on day 5. Although, the function of this molecule is not clear we were able to determine some calcium binding capacity using ⁴⁵Ca in blotted material. These preliminary data suggest a possible role at the initial phase of development.

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05-102

A COLD-INDUCIBLE GENE ISOLATED FROM DIAPAUSE EGGS OF *BOMBYX MORI* USING RNA DIFFERENTIAL DISPLAY: SEQUENCING OF THE CDNA AND ITS GENE EXPRESSION

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As an initial step to isolate functional molecules involved in maintenance and/or termination of embryonic diapause of *Bombyx mori*, we have performed RNA differential display using cDNAs prepared from diapause eggs, and then obtained a PCR product expressed much more in eggs exposed to 5°C than in eggs incubated at 25°C continuously. Using this PCR product (Dif-L2), a cDNA was cloned from a cDNA library made with eggs. The cDNA for Dif-L2 contained 2400 nucleotides and an ORF consisting of 678 amino acids, with both start and stop codons. Northern hybridization and RT-PCR experiments showed that an RNA with about 2.5 kb increased in diapause eggs exposed to 5°C for 10-20 days while it was at very lower levels in eggs incubated at 25°C, with the same ages. Although protein(s) similar to Dif-L2 were not yet found using data bank, Dif-L2 protein had a domain specific to proteins which were translocated into nucleus. These results suggest that the gene expression for Dif-L2 is induced by cold-treatment and that Dif-L2 functions within nuclei in *Bombyx* eggs.

05-104

QUINONE-TANNING IN THE EGG-SHELL OF THE FALSE MELON BEETLE, *ATRACHYA MENETRIESI* FALDERMANN (COLEOPTERA: CHRYSOMELIDAE)

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A newly laid insect egg is surrounded by two envelopes. The inner envelope is the vitelline membrane and the outer is the chorion or eggshell. In some species, the envelopes sclerotize after oviposition. Such a case was previously reported in dragonfly eggs (Kawasaki *et al.*, 1974). The dragonfly vitelline membrane, which is stabilized by intermolecular crosslinks in the form of di- and trityrosyl residues, sclerotizes further probably by the action of phenoloxidase, accompanied by a change in color from yellow to dark brown. In this paper, we report another example of the sclerotization of egg envelopes of the false melon beetle, *Atrachya menetriesi* Faldermann. The newly laid eggs of the chrysomelid beetles are yellow, and the color changes to dark brown after several h. We collected the eggs, and prepared their scleroprotein fractions. Then, the fractions were subjected to column chromatography with BioGel P-2 after being hydrolysed. Several phenolic compounds were found in the hydrolysates, and some of them were identified as the conjugates of 3,4-dihydroxyphenylacetic acid and L-cysteine, which were 5-S-cysteinyl-, 2-S-cysteinyl-, and 2,5-S,S-dicysteinyl-3,4-dihydroxyphenylacetic acids. The content of 5-S-cysteinyl-3,4-dihydroxyphenylacetic acid, the major compound, in the brown eggs was substantially lower than that in the yellow eggs. 3,4-Dihydroxyphenylacetic acid was detected as a female-specific compound in the whole bodies of the insects. These results suggested that quinone tanning took place in the eggs of the beetles, using 3,4-dihydroxyphenylacetic acid as the precursor of the tanning or sclerotizing agent. The egg envelopes are stabilized probably by the crosslinks formed from the quinone of 3,4-dihydroxyphenylacetic acid and cysteinyl residues in the proteins.

05-103

TIME INTERVAL MEASURING ENZYME FOR RESUMPTION OF EMBRYONIC DEVELOPMENT AND FUNCTION OF EGG-SPECIFIC PROTEIN IN THE SILKWORM, *BOMBYX MORI* H.Kai, N.Katagiri, M.Isobe¹ and S.Suwan¹
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EA4 is a DNA-dependent ATPase which has the capability of measuring a time-interval for resumption of embryonic development in diapause eggs of the *Bombyx* silkworm. PIN peptide regulates the EA4. We asked what is the mechanism by which EA4 measures time and PIN regulates the timer.

At 5 °C, EA4 activity was very low initially, but in 2 weeks the activity increased sharply and then rapidly fell. The period required to activate the enzyme was equivalent to that observed *in vivo* and was coincident with the chilling period indispensable for diapause termination. In mixing of PIN with EA4, the clock-run of EA4 was delayed to the period equivalent of PIN inclusion. PIN may hold the timer probably by forming a PIN-EA4 substructure. PIN was identified. The full sequence of PIN was 100% identical to a portion of amino acid sequence of Egg-Specific Protein (ESP); the 19th-56th amino acid residues of the ESP, in which the 1st-18th residues are likely to be a signal peptide and the 57th-58th residues (K-R) are possibly a cleavage site.

EA4 may originally be complexed with PIN, which resulted after processing from ESP. In winter cold, PIN and EA4 may dissociate from each other. The dissociation may cause EA4 to undergo conformational changes, resulting in a time-interval activation of the enzyme which is crucial for diapause termination.

05-105

RHYTHM OF PERIOD PROTEIN EXPRESSION IN MALPIGIAN TUBULES OF *DROSOPHILA MELANOGASTER*: EVIDENCE FOR HEAD-INDEPENDENT CIRCADIAN CLOCK

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Period (per) gene is part of the molecular feedback loop involved in circadian clock of *Drosophila*. *Per* is expressed rhythmically in the brain and appear to control the rhythms of locomotor activity and eclosion. We have found that *Drosophila* excretory epithelium (Malpighian tubules) express *per* protein (PER) rhythmically, even in brainless flies. Our initial studies of *per* expression in *Drosophila* were carried out on transgenic flies (XLG) (gift from Dr. J. Hall) which contain 99% of the *per* coding region fused to all of *E.coli lacZ* gene. XLG flies were shown to express the PER-reporter fusion protein rhythmically in relevant cells in the brain (R. Stanewsky and J. Hall, pers. comm.). The following observations will be reported: (i) Expression of PER in Malpighian tubules (MTs) is rhythmic, PER is first detected in the cytoplasm and then, at the end of dark phase, in the nucleus. (ii) MTs of *per⁰* flies do not express PER. (iii) Rhythm of PER in MTs continues in constant darkness. (iv) Rhythm of PER in MTs continues and phase shifts in response to shifted photoperiod in brainless flies. (v) PER expression in MTs is "turned on" at eclosion. MTs of insects in the pupal stage are largely inactive; interestingly, PER was not detected in MTs at any time during the day preceding eclosion. Thus, PER in MTs seem to be developmentally regulated, and its appearance may coincide with the onset of adult secretory functions.

In summary, our results suggest that PER in Malpighian tubules behaves very similarly to PER in lateral neurons of the brain: it cycles and translocates into the nucleus in the wild flies in LD and DD and is absent in *per⁰* flies. Moreover, the PER rhythm continues and phase shifts in brainless flies. These findings suggest that MTs may be the site of a self-contained circadian clock.

05-106

GROWTH AND DEVELOPMENT IN *TENEbrio MOLITOR* LARVAE CHRONICALLY EXPOSED TO GAS MIXTURES: VARYING O₂ PRESSURES IN N₂ AND He.

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This study was designed to examine the response of *Tenebrio molitor* larvae to different O₂ pressures during development, including two different hypoxic environments: 10% O₂ in N₂ and in He. Oxygen diffusion in He is about four times faster than in N₂. Larvae reared from eggs at 25°C and 70% RH, in four O₂ environments: hypoxia (10% O₂ in N₂ and in He; PO₂=74 Torr), hyperoxia (40% O₂ in N₂; PO₂=297 Torr) and normoxia (air; PO₂=155 Torr). Pupation of first larvae exposed to normoxia and hyperoxia started after day 125 post hatching, while in hypoxic atmospheres it began after day 160. 48% of the larvae survived the N₂ hypoxia; 76% survived in normoxia; 96% in hyperoxia; and 80% in 10% O₂ in He hypoxia. Survival differences were significant ($X^2=69.7$, $p<0.01$; initial $n = 128, 118, 93$ and 111 , respectively). Average body masses on day 180 after hatching differed and were $64.5 \text{ mg} \pm 11.6\text{SE}$, $143.6 \text{ mg} \pm 10.6\text{SE}$, $133 \text{ mg} \pm 9.7\text{SE}$ and $79.3 \text{ mg} \pm 4.3\text{SE}$ in N₂ hypoxia, normoxia, hyperoxia and He hypoxia ($n=25$ in each group, $p<0.01$). Growth curves fitted to larval body mass were similar between normoxic and hyperoxic treatments but had a 4-fold significantly higher developmental rate than in hypoxic groups.

Oxygen growth response to hypoxia may exist and be sensitive to oxygen pressures. Increased oxygen flux in He hypoxia was shown to increase survival.

05-108

THE SERINE PROTEINASE INHIBITORS IN COCKROACH INTESTINES

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There are only few insect species, which have proteinase inhibitors in intestine. The highest level of trypsin-like serine proteinases activity are determined in posterior midgut. There is not occurs digestion of proteins in the crop and anterior midgut. The thermostable serine proteinase inhibitors were discovered in crop and anterior midgut of the 6 cockroach species: *Blatta orientalis*, *Blattella germanica*, *Periplaneta americana*, *Nauphoeta cinerea*, *Blaberus craniifer*, *Gromphadorina* sp. Their activity was assessed by gelatin lysis of film for aerophoto after exposure to heat-treated extract (10 min in boiling water). The inhibitors suppress the activity of the bovine trypsin and enzymes in different levels as of the same species of cockroach as of some others. Therefore, the cockroaches of various species have the different composition of proteolytic enzymes and different structure of inhibitors. It is supposed, that inhibitors in the gut of irregularly feeding cockroaches promote food conservation in crop and anterior midgut and protect midgut epithelium from protease impact, particularly in starvation periods.

05-107

CHARACTERISTICS OF DIGESTIVE PROTEASES IN VARIOUS INSECT GUTS

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The nature of proteases in gut digestive fluid are different between insect order. It has been reported that some lepidopterous species mainly have trypsin like serine-proteases, orthopterous and hymenopterous species have chymotrypsin, and coleopterous species have cysteine-proteases. However only a few data have been useful. I presents here the differences of the nature of digestive proteases between some insect orders by using polyacrylamide gel analysis. Gut was dissected from insect abdomen in a pre-chilled saline on ice and centrifuged to take digestive fluid. The fluid was electrophoresed on 4% gelatin-10% SDS-PAGE gel. The gel was incubated with or without some kinds of protease inhibitors and stained with CBB dye. Subunits of protease can be seen as transparent bands in a blue background. When the gel is incubated with a protease inhibitor, inhibited bands are stained and fade into the background. For example, the main gut protease of *Chilo suppressalis* (Lepidoptera: Pyralidae) is serine-protease, so that it was inhibited by PMSF and corresponding bands on a gel were stained with CBB. On the other hand, that of *Lissorhoptrus orzohilus* (Coleoptera: Curculionidae) was not inhibited by serine-protease inhibitor, but inhibited by cysteine-protease inhibitor, iodine acetate, NEM, pHMB and leupeptin, therefore the main protease of this species is cysteine protease. I will show the results on the other species including Orthoptera, Diptera, Odonota.

05-109

NEW MIDGUT PROTEASE ACTIVITIES IN COLORADO POTATO BEETLE LARVAE

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The midgut of the Colorado potato beetle larvae, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) has mildly acidic pH, and cathepsin B, D, H and L have been reported to provide the major midgut proteolytic activities.

We have characterized a new endoprotease activity using the chymotrypsin-like specific substrate N-succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine-p-nitroanilide. This novel proteinase, with a pH optimum of 6.5-7.0, was not activated or inhibited by thiol compounds and cysteine specific proteinase inhibitors. Among several serine proteinase inhibitors tested, chymostatin resulted the most effective. Elastase-like activity, using the specific substrate N-succinyl-alanine-alanine-alanine-p-nitroanilide, was not detected.

The major exopeptidase found in the larval midgut was a leucine aminopeptidase activity with a pH optimum of 7.5. Carboxypeptidase A and B activities were not detectable by using hippuryl-L-phenylalanine and hippuryl-L-arginine as substrates, respectively.

05-110

PROTEINASES OF COLORADO POTATO BEETLE AND THEIR PLANT PROTEIN INHIBITORS

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Colorado potato beetle (CPB) larvae and adults digestive proteinases were separated by isoelectric focusing (IEF) in PAG and some bands with pI value of 4 to 10 were detected with use of gelatine layer of photofilm. The majority of bands were found to be inhibited by p-CMB. The cross-methods were used for the search of plant protein inhibitors active to CPB proteinases. The water-soluble proteins from bread wheat endosperm and potato tubers and leaves were applied on gels by wide strips and separated by IEF. The gelatine replicas (on photofilms) were obtained. The narrow pieces of replicas were developed by trypsin, chymotrypsin, subtilisin, ficin and wheat germinated grain cysteine proteinases for detection of inhibitors. The CPB proteinase samples were applied on IEF gels by wide strips and separated as well. The gelatine replicas were superimposed on IEF gels with CPB proteinases so that plant protein bands were perpendicular to proteinase bands. The proteinases digested gelatin of replicas as transparent bands narrowed or interrupted in points of intersection with bands of their inhibitors. The comparison of results of cross analysis with patterns of standard proteinase inhibitors have shown that some CPB proteinase bands are inhibited by wheat cysteine proteinase inhibitors and some others are inhibited by trypsin and chymotrypsin inhibitors from potato tubers and damaged leaves. It was found that potato varieties vary in capability to accumulate inhibitors of trypsin, chymotrypsin, subtilisin and elastase in damaged leaves. The resistant to CPB varieties Zarevo, Zov and Liza accumulated inhibitors in contrast to susceptible varieties.

05-111

INVESTIGATION OF PROTEIN INHIBITORS OF TRYPsin-LIKE PROTEINASES AND CALPAIN I AND CALPAIN II ACTIVITIES IN TISSUES AND ORGANS OF SILKWORM, BOMBYX MORI L.

S.M.Klunova, N.V.Tarassenko, E.Yu.Kolobova,
Yu.B.Philippovitch.

Laboratory of insect biochemistry, chair of organic and biological chemistry of Moscow State Pedagogical University, Moscow, Russia. The dynamics of protein inhibitor activity of trypsin-like proteinases was studied in haemolymph, carcass, fat body, fibroin and sericine divisions of silk glands in silkworm at the final stage of its larval development. Little changes of protein inhibitor activity were shown in haemolymph, whereas it markedly increased in the other tissues by the end of the 5th age and decreased practically to the initial level by the third day of the cocoon spinning. Five peaks of inhibitor activity differed for pI values was found by isoelectrofocusing both in fibroin and sericine parts of silk glands. We have found the high level of Calpain I and Calpain II activity both in fat body and in fibroin division of the silk glands. In this connection protein inhibitors of Ca^{++} -dependent neutral proteinases activity in the silkworm will be studied.

The role of the protein inhibitors of peptidohydrolases and the complex of proteolytic enzymes in nitrogen metabolism in tissues and organs of silkworm is discussed.

05-112

IN VIVO MANIPULATION OF AMINOPEPTIDASE AND CARBOXYPEPTIDASE ACTIVITIES IN THE MOSQUITO, ANOPHELES STEPHENSI LISTON (DIPTERA: CULICIDAE).

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Enzymes are considered targets for anti-parasites vaccines because of their functional importance. A carboxypeptidase-type enzyme in the tick (1) is the proposed Bm91 vaccine antigen and aminopeptidase is currently under trial as a vaccine against *Haemonchus contortus* (2). Aminopeptidase has also been implicated in mosquito refractoriness to *Plasmodium* parasites (3).

We have characterised two midgut enzymes of *Anopheles stephensi* which display characteristics typical of aminopeptidase M and carboxypeptidase A. Aminopeptidase appears to be synthesised in a form bound to the midgut membrane by a glycosyl phosphoinositol anchor, then cleaved into a soluble form, active in the lumen and associated with the peritrophic matrix. Carboxypeptidase also appears to have both membrane-bound and soluble forms.

Mosquitoes fed aminopeptidase inhibitor revealed that a possible "cascade" mechanism of synthesis exists. In addition, mosquitoes fed aminopeptidase or carboxypeptidase inhibitors seem able to compensate for inhibition of either peptidase, with increased synthesis of the other. When fed both inhibitors, aminopeptidase and carboxypeptidase activities were only partially reduced. This manipulation of activities has adverse effects on mosquito fecundity.

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(2) Smith, T.S. et al (1993). *Int. J. Parasitol.* **23**, 271-280.

(3) Feldmann, A.M. et al (1990). *Parasitol.* **101**, 193-200.

05-113

AEROBIC METABOLISM IN THE PAUNCH OF THE HIGHER TERMITE NASUTITERMES WALKERI

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The recent demonstration by Breznak and co-workers that the hindgut of both higher and lower termites, previously thought of as an anaerobic environment, contains a substantial amount of O_2 suggests that a significant amount of aerobic metabolism is taking place. We have been measuring in the hindgut the activities of the major enzymes of aerobic metabolism as well as the enzyme activities involved in the activation of potential substrates for energy such as glucose, xylose, pyruvate and acetate. The pyruvate dehydrogenase (PDH) complex and the tricarboxylic acid cycle are active in the hindgut. The activity of the PDH complex is of particular interest as it is inactive in the aerobic termite body tissues. This suggests that pyruvate produced in termite tissues by glycolysis is a major substrate for bacterial growth and energy. Pyruvate cannot be detected in the hindgut suggesting that pyruvate is metabolised as soon as it enters the hindgut. O_2 utilisation by the bacteria is extremely sensitive to variations in pyruvate concentration, much more so than other substrates such as glucose, xylose, acetate or alanine. In contrast, the enzymes of glucose activation in the hindgut are either absent (glucose phosphotransferase) or have low activity consistent with the absence of cellulase activity in the hindgut and suggesting that glucose is not a normal bacterial substrate in the hindgut. There is evidence that xylan, one of the components of hemicellulose, is metabolised both by the termite and its symbiotic bacteria as the enzymes of xylan metabolism are evenly distributed throughout the gut and that xylulokinase, a key enzyme in the activation of xylose, is active (30 nmol/termite/h) in the bacteria. The role of acetate in the hindgut is equivocal. Its high concentration (27 mM) suggests that it is not metabolised to any great extent, consistent with the traditional view of short chain fatty acids being the end products of anaerobic metabolism. However, the activity of acetyl CoA synthetase, the enzyme responsible for the activation of acetate, is 18 fold higher in the hindgut (213 nmol/termite/h) than in the body (12 nmol/termite/h), the expected site of acetate metabolism.

05-114

CROP EMPTYING IN RELATION TO FOOD TYPE IN THE CRICKET, *TELEOGRYLLUS COMMODUS* WALKER, AND THE COCKROACH, *PERIPLANETA AMERICANA* L.

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Crop emptying in herbivorous insects may determine the rate of digestion and absorption, as the emptying determines the rate of movement of food into the midgut and hindgut and may influence the interval between meals. Studies on acridids have indicated that crop emptying is under neural and/or endocrine control, but these studies have ignored the role that the morphology of the gut may play in crop emptying as well. The cricket has an unusual gut structure, in that the proventriculus is a muscular, cuticular-toothed lined organ and then connects into a very short midgut. The proventriculus acts as a second compartment for mixing of food, in addition to the crop, and is separated from the crop by a muscular sphincter. Rather than a single continuous flow-stir tank reactor (CSTR) (as defined by chemical reactor theory), the cricket has two CSTRs, followed by a short plug-flow reactor (PFR), the midgut. In contrast the cockroach, similar to acridids, has the single CSTR (the crop), followed by a relatively long PFR. Chemical reactor theory would suggest that the passage time of food would increase as more CSTR systems are present. Our work indicated that passage time of food depended upon the type of food ingested in both the crickets and cockroaches, with fresh lucerne passed from crop to midgut at nearly the same rate in the two insects, while rye grass passed more slowly through the cricket. This indicates that food type plays an important role in determining rate of crop emptying as previously shown for acridids. In addition, the presence of the CSTR (proventriculus) in the cricket does not necessarily lengthen passage time, implying that changes in gut peristalsis can influence the food movement such that the CSTR mimics a PFR.

05-116

EFFECTS OF SOYBEAN TRYPSIN INHIBITOR, GOSSYPOL AND TANNIC ACID ON THE DIGESTIVE PHYSIOLOGY AND GROWTH OF THE LARVAL *HELICOVERPA ARMIGERA* (HÜBNER)

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The effects of soybean trypsin inhibitor (STI), gossypol and tannic acid on the digestive physiology and growth of cotton bollworm *Helicoverpa armigera* (Hübner) were studied. Each of these three agents inhibited larval midgut proteolytic activity *in vitro*; STI was the most effective. When incorporated into an artificial diet, 0.84% (dry weight) STI significantly reduced the high alkaline trypsin-like enzyme activity by 18%; 0.3% tannic acid significantly decreased the low alkaline trypsin-like enzyme activity and total proteolytic activity by 22% and 18% respectively, and 0.3% gossypol had no significant effect on proteolysis. All these three agents markedly suppressed the growth of the larvae when incorporated into the diet in these ratios. The coexistence of STI and gossypol or tannic acid respectively declined the low alkaline trypsin-like enzyme activity by 34% and 66%, the high alkaline trypsin-like enzyme activity by 30% and 55%, and the total proteolytic activity by 30% and 66%. The coexistence of STI and tannic acid also reduced chymotrypsin-like enzyme activity by 53%. Both gossypol and tannic acid potentiated the larval growth inhibitory activity of STI, and this fact implicates the protease inhibitor gene engineered into the cotton plant with high gossypol and/or tannic acid content may achieve greater protection against the cotton bollworm.

05-115

EFFECT OF *Annona muricata* SEED EXTRACTS ON THE NUTRITION INDICES OF *Heliothis armigera* LARVAE

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Effect of *Annona muricata* seed extracts on the nutrition indices of the fifth-instar of *Heliothis armigera* larvae was carried out using artificial diet. The result indicated that the relative consumption rate was decreased, and consequently, the growth was inhibited. Interestingly it seems that in response to the toxic materials from the extracts, the larvae compensated by increasing the food assimilation, however this compensation was not enough to support the normal growth.

05-117

THE EFFECTS OF *DERRIS ELLIPTICA* ROOT EXTRACTS ON THE FOOD INTAKE, FOOD UTILIZATION, GROWTH AND OXYGEN CONSUMPTION IN THE FIFTH-INSTAR LARVAE *HELIOTHIS ARMIGERA* (LEPIDOPTERA: NOCTUIDAE)

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The effects of feeding artificial diets treated with various concentrations of *Derris elliptica* root ethanol extract or ether fraction extracts to the fifth-instar *Heliothis armigera* larvae are presented. The results showed that, in general, the growth and efficiencies of food use were significantly lower in the case of derris treated larvae than that of the controls, with the exception that the larvae treated with derris root ethanol extracts had significantly higher Approximate Digestibility (AD) values as compared to that of the controls. As expected, oxygen consumption rate was significantly lower on larvae treated with derris root extracts.

05-118

GLYCINE SECRETION AS A NOVEL STRATEGY OF INSECTS AGAINST CHEMICAL DEFENSE OF HOST PLANTS

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We found that a considerable amount of free glycine exists in the digestive juices of several leaf-feeding Lepidopteran larvae. The concentration of glycine has very clear correlation with the protein denaturing activity of the host plant. We found that the leaves of the privet tree, *Ligustrum obtusifolium*, have extremely strong protein denaturing activity enough to make a protein innutritive. The denaturing activity closely resembles those of polyphenolics, general defense agents widespread throughout plants. The species which feed on this host plant have an extremely large amount of glycine in their digestive juice. The concentration reaches 50-60 $\mu\text{moles/ml}$ (0.4%) in *Dolbina tancrei* (Sphingidae) and *Brahmaea wallichii* (Brahmaeidae), both of which are specialists to the privet tree. We demonstrate that free glycine can completely inhibit the denaturing activity of the privet leaf in vitro, and that free glycine can prevent the protein from becoming innutritive using bioassay.

This is the first demonstration of the fact that such a simple molecule as a glycine can work as, and is actually used by herbivores as a counteracting agent against denaturing activity of plants. We believe that this is a good example of chemical adaptation and coevolution between plants and herbivores.

05-120

THE EFFECT OF AMINO ACIDS ON SODIUM UPTAKE BY THE MIDGUT OF THE CECROPIA LARVA.

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It has for some time been assumed that sodium uptake by the larval midgut of some herbivorous insects takes place via the potassium/amino acid co-transporter, which has a higher affinity for sodium than for potassium at low concentrations of the ions (Hanozet et al., 1992). Chamberlin, (1990), measured sodium uptake in the midgut of *Manduca sexta* larvae in the presence of all the naturally occurring amino acids. Recently, it has been shown that sodium can be taken up by the Cecropia larval midgut without any amino acids present in the bathing solutions (Nedergaard and Wolters, in press). The potential difference across the midgut, normally 100 - 160 mV, is favorable for sodium uptake, but can account for only about half of the uptake. The presented results show the effects of an amino acid, leucine, on the sodium uptake by the larval midgut. The presence of leucine increases the sodium flux to nearly the double of the flux in the absence of amino acids measured across isolated tissue. A similar effect is also observed using brush border membrane vesicles prepared from the Cecropia midgut tissue.

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05-119

INDUCTION OF A K^+ -DEPENDENT LEUCINE TRANSPORT IN *XENOPUS LAEVIS* OOCYTES BY INJECTION OF mRNA ISOLATED FROM THE MIDGUT OF THE SILKWORM *BOMBYX MORI*.M. Casartelli MG Leonardi, P Parenti¹, B Giordana

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Lepidopteran larval midgut presents two different cell types: the goblet cells, responsible for ion regulation, and the absorptive columnar cells.

The proton pump on the luminal membrane of the goblet cells, associated to a $\text{K}^+/\text{2H}^+$ antiporter, generates the high lumen-positive electrical potential difference (100-150 mV) and the steep luminal K^+ concentration (150mM) which drive the absorption of amino acids by the K^+ /amino acid symporters localized on columnar cell microvilli.

The injection of poly(A)⁺ mRNA isolated from *Bombyx mori* midgut into *Xenopus laevis* oocytes determined the appearance, 3-5 days after injection, of a K^+ -dependent uptake of radiolabelled L-leucine. The K^+ -dependent leucine transport was detectable with an external cation concentration of 20 mM and represented a 50% increase of basal leucine uptake, which in non-injected and water-injected oocytes was Na^+ -independent.

Since the K^+ /amino acid transport of neutral amino acid is strongly activated by the electrical potential, the membrane potential of mRNA-injected oocytes was modulated and K^+ -dependent leucine uptake measured. The expressed amino acid uptake was completely inhibited by a depolarising K^+ concentration (100 mM) or by the addition of 0.5 mM ouabain in the incubation medium.

The affinity of the transport for its substrates was also determined.

05-121

MECHANISM OF ACTION OF CCRF-LIKE DIURETIC PEPTIDE: STIMULATION OF TWO INTRACELLULAR SIGNAL PATHWAYS

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CCRF-like diuretic peptide (CCRF-DP) isolated and synthesized from *Culex* has dose-dependent effects on transepithelial voltage (V_t) in isolated perfused Malpighian tubules of *Aedes aegypti*. At low doses ($<10^{-8}$ M) CCRF-DP causes only a depolarization of V_t together with a drop in transepithelial resistance (R_t) without a change in the short-circuit current (I_{sc}). These electrophysiological effects are identical to those of leucokinin (J. Membr. Biol. 132, 63, 1993) and indicate an effect on the paracellular pathway in general, and on shunt Cl conductance in particular. At concentrations higher than 10^{-8} M CCRF-DP causes a depolarization followed by a hyperpolarization of V_t together with a drop in R_t and an increase in I_{sc} . These effects are identical to those of mosquito natriuretic peptide (NIPS 2, 171, 1987) and indicate an effect on the transcellular pathway in general, and on active transepithelial Na transport in particular. In separate experiments the effects of low doses of CCRF were duplicated with calciomycin (A23187), an ionophore of calcium. The effects of high doses of CCRF-DP were mimicked with the second messenger cyclic AMP. Thus it appears that the diuretic responses of Malpighian tubules to CCRF-DP are graded and additive through dose-dependent recruitment of different receptors and/or intracellular second messenger pathways. (Supported by NSF IBN 9220464)

05-122

MOULT CYCLE DEPENDENT EXPRESSION OF A Cl^- CHANNEL IN AN INSECT EPITHELIUMI. Watson, S. Caveney, D. Churchill¹

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Video microscopy and whole-cell patch-clamp recording were used to monitor cell volume changes of newly-moulted epidermal (NME) cells of the flour beetle larva *Tenebrio molitor* challenged by an hyposmotic stress and to record any resulting currents. Cells reached maximal volume (14% above normal) four minutes after treatment with an hyposmotic solution (80% osmolality of normal saline) and then underwent a regulatory volume decrease (RVD) that stabilized within fourteen minutes. Application of the Cl^- channel blocker, diphenylamine-2-carboxylate (DPC, 200 μM), during hyposmotic stress blocked cellular RVD and resulted in a further volume increase (25% above normal). By varying the internal and external Cl^- concentrations, it was shown that the reversal potentials of measured swelling-activated and non-swelling currents closely followed the calculated Cl^- equilibrium potentials with a linear regression slope of 0.82. Application of DPC during current recordings reduced current strength by 60%, which was mostly reversible upon washing. These data suggest that this current is the result of the efflux of Cl^- from the epidermal cells during osmotic stress. Mid-instar cells, which are only half the volume of NME cells, have 25% of the inducible Cl^- current, implying that the number of epidermal Cl^- channels depends on the stage in the moult cycle. This osmo-sensitive Cl^- channel may be involved in the regulation of cell volume during the moult cycle.

05-124

EXTRACELLULAR MATRIX GLYCOPROTEINS IN THE MOSQUITO MIDGUT

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Insect extracellular matrices (ECM) are important but poorly understood. The molecular composition and functional roles of ECM molecules have been investigated in very few studies (for review see Lane *et al.*, 1996). Midgut epithelial cells rest on a continuous basement membrane or basal lamina (BL), which is thought to be involved in such vital cell processes as polarity, epithelial integrity, cell adhesion, selective permeability and tissue development. We have undertaken a preliminary characterization of the BL in the midgut of the mosquito, *Anopheles stephensi*. Commercially available antibodies raised against mammalian ECM molecules have been used to identify collagen IV, elastin, fibronectin and laminin. In immunofluorescence assays (IFA) all four molecules were localised to the BL of the midgut epithelial cells. Dot blots of whole gut homogenates and extracts of *An. stephensi* and *An. gambiae* cell cultures confirm the presence of the same four BL components. Western blots of cell culture extracts suggest that mosquito laminin is a polymer of three chains, as in other invertebrates and vertebrates, with subunits of approximately 70 kDa, 100 kDa and 110kDa. Collagen and fibronectin have also been visualised by SDS-PAGE and Western blots and work is continuing on the molecular characterization of these ECM molecules.

Lane, N. J., Dallai, G., and Ashurst, D. (1996) Structural macromolecules of the cell membranes and the extracellular matrices of the insect midgut. In Billingsley, P. F., and Lehane, M. J. (editors). *Biology of the Insect Midgut*. Chapman & Hall.

05-123

TASTE RECEPTION MECHANISMS IN *Protophormia terraenovae*: EVIDENCE OF AMILORIDE-SENSITIVE AND INSENSITIVE RECEPTOR SITES.

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In various vertebrate species an amiloride-sensitive cation conductance has been shown to be involved in NaCl as well as sugar reception mechanisms (Cummings *et al.*, 1993; Ossebaard and Smith, 1994). Studies on salt and sugar reception in insects have been particularly intense on the gustatory apparatus of blowflies, but no information is yet available about amiloride effects on membrane receptor sites. We thus investigated the effects of amiloride on the responses to various taste stimuli in the blowfly species *Protophormia terraenovae*, with the aim of ascertaining whether an amiloride-sensitive cation conductance is also present in the chemosensory systems of insects. Electrophysiological experiments show that: (a) spike firing frequency of the "salt" cell in response to NaCl , sucrose, bovine serum albumin (BSA) and L-alanine (the C-terminal amino acid of the BSA molecule) is not influenced by the addition of amiloride (100 and 500 μM); (b) addition of amiloride does not influence the spike firing frequency of the "sugar" receptor cell in response to sucrose, but depresses the response to BSA and L-alanine; (c) the response of the "water" cell to all tested stimuli is not affected by addition of amiloride at 100 μM , but is depressed at 500 μM ; (d) pure amiloride, both at 100 and 500 μM , generally evokes a weak response from the "salt" cell. According to our results the "salt" cell seem to have no amiloride-sensitive receptor sites. Instead, the "sugar" cell apparently bears an amiloride-sensitive receptor site, distinct from the pyranose site, which appears to mediate the stimulatory effectiveness of BSA and L-alanine on this cell.

Cummings T. A., Powell J. and Kinnamon S. C. (1993) *J. Neurophysiol.*, 70:2326-2336.

Ossebaard C. A. and Smith D. V. (1994) *Chem. Sens.*, 6: 197-214.

05-125

ACTIVATION OF PROTEASES IN THE HEMOLYMPH OF THE AMERICAN COCKROACH (*PERIPLANETA AMERICANA*) AS A CONSEQUENCE OF STRESSS. Liu¹, R.A. Cook², and J.E. Steele¹

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In the *American cockroach* the induction of stress, such as that resulting from the application of an insecticide, causes large, generally more than 25-fold, increases in the total protease activity of the hemolymph. The increase in the activity of the protease could be blocked by the application of a neck ligature prior to treatment with the insecticide.

Partial purification of the proteases was achieved by size exclusion chromatography of whole hemolymph on Sephadex G-100. All of the activity was eluted in a broad peak. The protease containing fractions were resolved using DEAE anion exchange chromatography and shown to be a mixture of at least six proteases. Two of the proteases were selected for further purification. The use of the insulin B-chain as a substrate showed that each of the proteases was clearly different in its substrate specificity.

Financial support for this research was received from Natural Sciences and Engineering Research Council of Canada (J.E.S.).

05-126

ISOLATION, BIOCHEMICAL AND FUNCTIONAL PROPERTIES OF MULTIPLE FORMS OF NADP-DEPENDENT SORBITOL DEHYDROGENASE FROM LARVAE OF SILKWORM, *BOMBYX MORI* L.

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Four forms of NADP-dependent sorbitol dehydrogenase (SDH) were purified to homogeneous state using affinity chromatography on Cibacron blue Sepharose and preparative gel electrophoresis. Two forms of enzyme from hemolymph (SDH1 and SDH2) catalyse glucose reduction to sorbitol at pH 6,3-6,5 while two other forms from midgut (SDH3 and SDH4) more active in revers reaction at pH 8,5-10,0 that correspondent to physiological pH in silkworm tissues. SDH1 and SDH2 are tetramers (Mr=30000 and 96000) while SDH3 and SDH4 are octomers (Mr=162000 and 138000). The forms of enzyme which consist of identical subunits have considerable analogy in amino acid composition. According to Km values the affinity of octomer forms to sorbitol is higher but their affinity to cofactor is lower than of tetramer forms. Kinetic properties of SDH forms are function of oligomerization.

05-128

Effect of a Trehalase Inhibitor, Validoxylamine A, on Three Species of Flies

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Effects of a trehalase inhibitor, Validoxylamine A (VAA), on the activity and haemolymph components were studied in three species of flies, *Musca domestica*, *Boettcherisca peregrina* and *Calliphora nigribarbis*. Haemolymph components were analyzed by NMR spectrometry. Overall activity of the housefly was suppressed by VAA injection (2.5 µg/fly) and 30% of them died in 2 days. The activity of the fleshfly also decreased but without death (2 µg/fly). VAA injection (5 µg/fly) acted, however, stimulatory rather than suppressively on the blowfly flight. Trehalose concentration in the haemolymph rose by VAA injection in a day to 2.6, 3.3, and 3.7 times in *M.domestica*, *B.peregrina* and *C.nigribarbis*, respectively. The trehalose accumulation seemed to be caused by inhibition of trehalose metabolism and by active synthesis of the trehalose from glucose which was not affected by VAA injection. The result that inhibition of trehalose metabolism did not evoke the reduction of the flight activity in the blowfly, suggests the use of alternative energy source in its body.

05-127

BIOCHEMICAL FEATURES OF THE TWO ADH ISOZYMES OF THE MEDFLY *CERATITIS CAPITATA*.

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The best characterised alcohol dehydrogenase (ADH; alcohol: NAD⁺ oxidoreductase; EC 1.1.1.1) from a non-Drosophilid insect is that of the Mediterranean fruit fly *Ceratitis capitata* (medfly, Diptera: Tephritidae). Like all *Drosophila* ADHs the enzymes of *C. capitata* belong to the short-chain dehydrogenase protein family, including several NAD-dependent dehydrogenases, which are characterised by a short polypeptide chain (~250 aa residues) and the absence of Zn for activity.

Biochemical-genetic studies showed that two medfly isozymes (ADH-1 and ADH-2) are encoded by two tightly linked genes (0.49 cM) on the left arm of the 2nd chromosome (Malacrida et al., 1992, *Biochem. Genet.* 30: 35-48) suggesting that the proteins are the product of gene duplication with divergence. In fact, the ADH isozymes differ in several features, such as pI, tissue localisation and development (Gasperi et al., 1992, *Biochem. Genet.* 30: 289-304). The two peptides, as determined by the nucleotide sequences of the corresponding cDNAs, show ~80% identity to each other, while they are ~40% identical to the *D. melanogaster* ADH peptide (Brognia et al., in prep.).

The catalytic properties of the two ADH isozymes have been determined on purified preparations, using primary and secondary alcohols as substrates and NAD⁺ as a coenzyme. As for the *Drosophila* enzyme, the medfly ADH-2 shows the highest activity for secondary alcohols, while the affinity of ADH-1 is highest for primary alcohols; moreover the affinity of ADH-1 for NAD⁺ is ten times higher than that of ADH-2.

Analysis of conformational changes during the interaction of each isozyme with substrates and the co-factor are in progress, using circular dichroism spectroscopy.

05-129

Effects of a Trehalase Inhibitor on the Haemolymph Energy Sources for Flight in the Locust, *Locusta migratoria*.

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Administration of validoxylamine A (VAA), a specific trehalase inhibitor, evokes various abnormality in insects. The cause of the abnormality is thought to be the stop of energy supply through the inhibition of hydrolysis of haemolymph trehalose. In *Periplaneta americana* which uses trehalose as the main fuel, the flight as well as oocyte development is inhibited by the administration. In the present paper, we examined the effect of VAA on the utilization of energy source for flight in the locust which has been known to switch the fuel from trehalose to lipids during persistent flight.

The trehalase inhibition in the flight muscle and an elevation of the haemolymph trehalose concentration occurred after the administration of VAA as observed in other insects. However, flight performance of the locust was not affected by the administration. After 1 hr of flight, the trehalose concentration in the haemolymph decreased and the lipid concentration increased in untreated locusts. In VAA treated locusts, both trehalose and lipid concentrations increased. These results may suggest that the elevation of the lipid concentration is caused by the limited supply of fuel from the inhibited trehalose metabolism, but not by the low concentration of trehalose in the haemolymph.

05-130

PROTECTIVE ROLE OF ALLOSTERIC EFFECTORS AND TREHALOSE ON THE THERMAL DENATURATION OF GLYCOGEN PHOSPHORYLASE B FROM THE FAT BODY OF LARVAL *Manduca sexta*.

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The allosteric activator, AMP, increased the thermal stability of glycogen phosphorylase b purified from the fat body of larval *Manduca sexta* in a dose-dependent manner. Thus, 20 mM AMP increased the half-time for thermal inactivation ($T_{0.5}$) by 3-fold at 60°C. IMP, which is also an allosteric activator was not able to increase the enzyme's thermal stability - IMP decreased the allosteric activation of the enzyme by AMP and also decreased the thermal protection conferred by AMP. The allosteric inhibitor ATP, which binds to the same site as AMP, was also able to increase the thermal stability of the enzyme, however with less efficiency than AMP. The substrate inorganic phosphate was able to increase the thermal stability, while the substrate glycogen was not. Organisms and cellular systems that are required to adapt to stress conditions, such as high temperature, respond by concentrating one of several organic solutes such as sugars, polyols or methylamines. Among these osmolytes are glycerol and trehalose, two polyols that are present in insect hemolymph. Glycerol at 600 mM increased 1.5-fold the $T_{0.5}$ for thermal inactivation of phosphorylase, while trehalose at the same concentration increased the $T_{0.5}$ 4-fold. Interestingly, in the presence of 20 mM AMP and 600 mM trehalose, 90 % of the enzyme activity remained after 20 minutes at 60°C. Supported by NIH grant GM 51296

05-132

IDENTIFICATION, PURIFICATION AND CHARACTERIZATION OF EPIDERMIS-ORIGIN HEMOLYMPH PROTEIN IN *GALLERIA MELLONELLA* L.

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Last instar larval integument of *Galleria mellonella* L. was *in vitro* cultured in culture medium including [³⁵S]-culture medium. Epidermis-origin hemolymph protein(EDHP) was identified by electrophoresis and autoradiography and purified from the hemolymph of last instar larvae by anion exchange chromatography, gel permeation chromatography, and chromatofocusing chromatography. EDHP is composed of monomer with apparant molecular weight of 47 kDa. EDHP was immunologically confirmed to be of epidermis-origin using antibody against it. Quantitative change and presence of EDHP in other organs were also investigated.

05-131

Characterization and Purification of Lysozyme in Haemolymph of the Larvae of *Lucilia illustris*

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Lysozyme is one component of the humoral defence system of the insects. Lysozyme in haemolymph of injured larvae of *Lucilia illustris* was identified and characterized with acidic electrophoresis and inhibition zone assay. Lytic activity against cell walls of Gram positive Bacteria shows similar effect in *Bacillus megaterium* and *Micrococcus luteus*. Lysozyme activity from injured haemolymph was higher than that of naive haemolymph. this enzyme was comparatively stable for pH and heat treatment, but heat stability was decreased drastically over 60°C. Lysozyme has been purified from injured haemolymph by reverse-phased FPLC.

05-133

STRUCTURAL CHARACTERIZATION OF THE SILKWORM HEMOLYMPH PROTEINS SEPARATED BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

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Hemolymph proteins from the 5th-instar larvae of the silkworm, *Bombyx mori*, N601 • 2 x C602 • 3 variety reared on an artificial diet were separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Totally 264 protein spots were identified on the 2D-PAGE gel. These proteins were electroblotted from the gel to a polyvinylidene difluoride membrane by Western blotting and detected by Coomassie blue staining. Among them, the sequenceable amounts of 53 proteins were subjected to gas-phase sequencing with an Applied Biosystems sequencer (477A). The N-terminal amino acid sequences of 29 proteins could be determined and then compared with those of proteins compiled in the Swiss-Prot database. The N-terminal sequences of only 7 proteins were consistent with the previously determined sequences of the silkworm hemolymph proteins such as antichymotrypsin (Takagi *et al.*, 1990), antitrypsin (Narumi *et al.*, 1993), juvenile hormone-binding protein (Kurata *et al.*, 1994) and three 30K proteins (Sakai *et al.*, 1988). The sequence of a protein with Mr 59 and pI 5.3 was similar to that of the α -lactalbumin (Brew *et al.*, 1970; Addeo, 1976; MacGillivray *et al.* 1979). The 2D-PAGE patterns of proteins with Mr 30-33 kD and pI 5.3-6.5, and Mr 26-31kD and pI 5.1-5.3 considerably varied depending on the individual. The amount of the silkworm SP-1 protein with Mr 80 kD was different between female and male larvae, as described by Izumi *et al.* (1980).

05-134

A SPECIFIC PEPTIDE PRODUCED DURING ADULT DIAPAUSE OF THE LEAF BEETLE, *GASTROPHYSA ATROCYANEA*H. Tanaka, K. Suzuki, Y. Hayakawa¹, K. Sato² and M. Kurihara

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Haemolymph proteins in adults of the leaf beetle, *Gastrophysa atrocyanea* have already been examined throughout the periods of pre-diapause, diapause, and post-diapause. A diapause-associated protein appeared at diapause initiation and disappeared at the end of diapause. On the contrary, an active phase-associated protein appeared at pre-diapause and post-diapause. In the present study, we paid particular attention to low molecular weight of proteins of the leaf beetle. As a result, a peptide appeared at diapause and disappeared at pre- and post-diapause. The mol. wt of this peptide was about 4.5 kDa. To isolate this specific peptide, we used the methods of disc preparative SDS-PAGE, gel exclusion HPLC and reversed HPLC. Some properties of this diapause-specific peptide were investigated to understand control mechanism of adult diapause. Then the N-terminal amino acids sequence for the first 34 residues was determined.

05-136

CONCENTRATION OF TREHALOSE AND ALANINE IN HAEMOLYMPH OF OVERWINTERING LARVA, *ENOSIMA LEUCOTAENIELLA* (R.) (LEPIDOPTERA: PYRALIDAE)M. Goto¹, K. Suzuki, M. Sakai²

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Diapausing larvae collected in October were incubated for 30 days at seven different temperatures (0 to 30°C). Trehalose which is dominant substance in haemolymph was high level at lower temperature and reached a maximum at 5°C. Glycogen contents in body were almost inversely proportional to trehalose concentration and reached a minimum at 0°C. More than 29 kinds of free amino acids were detected in haemolymph by using high speed amino acid analyzer. The concentration of alanine in the haemolymph of the overwintering larvae which had experienced the regimen of low temperature at 0°C was 1.8 fold high level compared with the larvae having experienced the regimen at 5°C. From September to January, trehalose concentration increased with decreasing glycogen, however, in February, both trehalose and glycogen level decreased. These results suggest that the high concentration of alanine might make an important role in glycogen decrease at low temperature below 5°C.

05-135

EFFECT OF CHILLING PERIOD ON PUPAL DIAPAUSE TERMINATION OF FLESH FLY, *BOETTCHERISCA PEREGRINA*A. Moribayashi, J. D. Wells, T. Hayashi, H. Kurahashi, N. Agui
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By using diapausing pupae of the flesh fly, *Boettcherisca peregrina*, we examined effect of chilling which may provide clues to the termination of diapause and developmental fate to adult. The fly enters to diapause at pupal stage under a short day condition (11L:13D) and low temperature at 20°C. Diapausing pupae were kept for 2 months at 20°C and then held at 4°C (chill treatment) for various duration between zero and 48 weeks before transfer to adult-developing condition at 27°C. 70–89% adult emergence occurred in pupae chilled for zero or 3–15 weeks, with the highest rate observed in those chilled 12 weeks. Relatively low emergence occurred in pupae chilled 1–2 or ≥ 20 weeks.

Distribution of adult-emergence days was determined for pupae with and without chilling. Pupae given no chilling emerged during a period of 59 days after transfer to 27°C. Pupae kept at 4°C for 12 weeks emerged during a narrow range of 23 days. All pupae that failed to emerge were dissected and confirmed to be dead. From these results we discuss about a relationship between chilling and diapause termination during pupal-adult development

05-137

DIFFERENCES OF COLD-HARDINESS BETWEEN THE SILVER Y MOTH, *AUTOGRAPHA GAMMA* (L.) AND THE ASIATIC COMMON LOOPER, *A. NIGRISIGNA* (WALKER).

J. Kaneko

Cold-hardiness of the silver Y moth, *Autographa gamma* on the stage of eggs, larvae, and pupae was compared with that of the Asiatic common looper, *A. nigrisigna* at the condition of 0°C, 100% R.H.

In each stage, both species individuals nearly all died after 10 to 30 days chilling without conditioning (10°C for 1 day and 5°C for 5 days). However, conditioned individuals survived more chilling days in both species, and this change was remarkable in the silver Y moth. Conditioned third instar larvae of silver Y moth survived 120 days chilling at rate of 1.4%. Contrary to this, none of conditioned individuals of *A. nigrisigna* survived 90 days chilling.

05-138

ICE-NUCLEATING ACTIVE AGENTS IN LARVAE OF THE RICE STEM BORER, *CHILO SUPPRESSALIS* WALKER (LEPIDOPTERA: PYRALIDAE)H. Tsumuki, M. Hirai¹

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Hibernating larva of the rice stem borer, *Chilo suppressalis* was a freezing tolerant insect. The larvae produced ice-nucleating active agents (INAs) in the muscle during hibernation. The production of the endogenous INAs was accelerated by low temperatures. They were sensitive to protease, stable at at least 50 °C for 5 min, and active over a broad pH range and after delipidation treatments. Consequently their chemical properties are more similar to those of the exogenous INAs of *Fusarium moniliforme* var. *subglutinans* isolated from the gut of the non-diapausing larvae rather than those of bacteria.

After differential centrifugation of the muscle homogenate, the crystallization temperature of the microsomal fraction was the highest among the all fractions. A small part of the muscle could raise the crystallization temperature of the haemolymph. These results suggest that the endogenous INAs are present on the outside of the muscle cell membrane and induce extracellular freezing by the same mechanism as that in other insects which possess INAs in the haemolymph.

05-140

RELATIONSHIP BETWEEN PTERIDINE METABOLISM AND SEASONAL COLOR DIMORPHISM IN THE SCORPION FLY, *PANORPA JAPONICA* THUNBERG

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The scorpion fly, *Panorpa japonica*, displays a seasonal color dimorphism by changing from black in spring to yellow in summer in the adult state.

The yellow pigment was identified as sepiapterin, while the black one was proved to be melanin. The quantities of these pigments in the integument of the mature fly varied depending upon the color dimorphism of the insect.

The combination of the morphological and biochemical approaches shows that sepiapterin is characteristically located at the pigment granules in the integument of the insect.

Various pteridines, neopterin, biopterin, isoxanthopterin and 7-oxo-biopterin, were also isolated in the adult scorpion fly besides sepiapterin.

The content of various pteridines and the activities of various enzyme related to pteridine biosynthesis (GTP-cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase and sepiapterin reductase) were measured at several stages after emergence of both yellow type fly and black one.

The results suggested that GTP-cyclohydrolase I is a key enzyme in the relationship between pteridine metabolism and seasonal color dimorphism.

05-139

BLUE PIGMENT BINDING PROTEINS FROM CABBAGE WHITEBUTTERFLY, *ARTOGEIA RARPAE* (LEPIDOPTERA)

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Three blue pigment binding proteins (BPs) were identified from the prepupal haemolymph by native polyacrylamide gel electrophoresis using Prep Cell. Two BPs were purified from the larval haemolymph of *Artogeia rapae*, and the physicochemical properties and changes during development were examined.

During last instar larval and prepupal stages, BP1 was maintained highly in haemolymph but disappeared after prepupal stage, whereas BP2 increased drastically in the prepupal haemolymph and showed maximum in pupal stage. And minute amounts of BP3 could be detected in the prepupal stage.

Antiserum against BP1 did not show cross-reactivity with BP2 in immunodiffusion test. BP1 was a major protein of extract from wing, and egg contained both of BP1 and BP2. BP1 is believed to function as storage proteins and insect coloration.

05-141

PROTEIN METABOLISM IN HONEY BEE NURSES

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When is a bee able to nurse? To determine this ability within the polyethism schedule of honey bees we injected C14-phenylalanine as a tracer into the haemolymph of differently aged bees. Then we estimated the amount of incorporation into protein of the abdomen, of the thorax and of the head, the latter mainly containing the hypopharyngeal glands (brood food glands). We recorded the behaviour of sister-bees of the same age in an observation hive. Brood nursing was defined when a bee inserted with her head into a broodcell for 1 to 20 seconds. Donating food by trophallaxis was defined when a bee held her tongue between the mandibles of the observed bee for more than one second.

Two hours after injection, in almost all investigated bees the greatest amount of phenylalanine was incorporated into the abdominal protein, whereas the smallest amount was measured in the thoraces.

We found brood nursing activity until an age of somewhat more than 2 weeks, but protein synthesis in the head could remain high until the 4th week. In different series of experiments the moment of maximum incorporation activity in the hypopharyngeal glands could vary. At the very end of their life this activity always decreased.

The fact that bees can synthesize so much protein even at an age when they do no longer feed brood could be explained by the synthesis of enzymes (Knecht and Kaatz, 1990, *Apidologie* **21**:457-468) and might indicate the synthesis of proteinaceous food that is given to other very young bees or foraging bees (Crailsheim, 1992, *J. Comp. Physiol. B* **162**: 681-689). Food was seen to be donated more often during this later period.

05-142

AMINO ACID METABOLISM IN HONEY BEE WORKERS AND DRONES

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Amino acids labelled with ^{14}C were injected into the haemolymph of various aged honey bee workers and drones with known amino acid concentrations. For 4 hours the bees were free to move and feed in a 20 ml translucent vial. Air was pumped through this vial and bubbled through Carbosorb (a CO_2 absorber from Packard). Four hours later, we determined ^{14}C incorporated into protein, remaining in the haemolymph, and absorbed in Carbosorb.

Compared to phenylalanine and leucine, proline was the predominant amino acid in the haemolymph (1:40 -1:20) and was subjected to oxidative metabolism even more pronounced. Dimensionally similar results could be seen for both sexes and all age classes investigated.

The incorporation of the injected amino acids into protein was generally fastest in young drones and dimensionally similar for all 3 amino acids. The greatest ratio was observed in 5 day old workers between leucine and proline; it was 1:5.

More than 50% of all amino acids in the haemolymph was proline. Although this amino acid plays a minor role in the total energy balance of bees compared to carbohydrates, its relatively high oxidative metabolism indicates its importance and outstanding role.

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05-143

MOLECULAR CLONING OF cDNA FOR HYPOPHARYNGEAL GLAND PROTEINS, AND A GENE EXPRESSING SELECTIVELY IN THE MUSHROOM BODY OF THE WORKER HONEYBEE (*APIS MELLIFERA* L.)

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Age-dependent role change is a characteristic feature of the honeybee, *Apis mellifera* L. society. The role of worker bees changes with age; young workers nurse brood by secreting and feeding bee milk, whereas older ones forage for nectar and process it into honey. Physiological changes occur in some organs according to the age and/or role change of the worker bee. We showed that three major proteins synthesized specifically in the hypopharyngeal gland of the nursebee are bee-milk proteins, while a major protein synthesized in the forager-bee gland is an α -glucosidase, indicating that the gland has two distinct differentiation state depending on the age-dependent role change (1).

Here, we isolated and analyzed cDNAs for these proteins. The results indicated that three bee-milk proteins share high sequence similarity with each other and those of RJP57-1 and RJP57-2 that are reported by Hanes and Simuth (2), indicating that they belong to a protein family. Honeybee α -glucosidase was found to share 70 % sequence similarity with possible *Drosophila* maltase gene product.

To investigate the molecular basis to cooperate the physiological conditions and the neural network of the brain of the worker bee, we searched for genes expressing selectively in the mushroom body of the worker bee brain by Differential Display as mushroom body is supposed to be a center to integrate sensory and motor neurons. One clone pMB5 was subcloned and found to express specifically in the large-type Kenyon cells of the mushroom body, indicating that the function of the gene is needed for a specific type of Kenyon cells.

References

1. Kubo, T., et al. *J. Biochem.* **119** 291-296 (1996)
2. Hanes, J. and Simuth, J. *J. Apic. Res.* **31**, 22-26 (1992)

05-144

PURIFICATION OF ENDO- β -1,4 GLUCANASE COMPONENTS FROM A LOWER TERMITE *RETICULITERMES SPERATUS*H. Watanabe, M. Nakamura, G. Tokuda¹, I. Yamaoka¹, and H. NodaNational Institute of Sericultural and Entomological Science, Tsukuba, Owashi, 305 Japan - ¹Faculty of Science, Yamaguchi University, Yamaguchi, Yamaguchi 753-01, Japan.

Two endogenous endo- β -1,4-glucanase components (YEG1 and YEG2) were purified and characterised from the lower termite, *R. speratus* using Superdex 75 molecular filtration and hydroxylapatite chromatography. Using a whole body extract prepared from workers and soldiers, one β -glucosidase and one endo- β -1,4-glucanase peak were obtained on chromatography with Superdex 75 with ammonium acetate buffer (0.3 M, pH 5.0). The endo- β -1,4-glucanase peak separated into two components (YEG1 and YEG2) on hydroxylapatite chromatography using a linear gradient of potassium phosphate (2-500 mM, pH 5.3). YEG1 and YEG2 had M_r of 45,900 and 45,000, respectively, on SDS-PAGE. Both components had an optimal pH of 6.0, and an optimal temperature of 50°C and were stable at 40°C for at least 30 min. YEG1 and YEG2 showed high activity against sodium carboxymethylcellulose (CMC), 73.6 units/mg protein for YEG1 and 83.4 units/mg protein for YEG2. YEG1 did not hydrolyse cellotetraose or cellotriose, while YEG2 hydrolysed cellotetraose and cellotriose. Both YEG1 and YEG2 hydrolysed cellopentaose to cellotriose and cellobiose, but neither component hydrolysed cellobiose. Cellobiose was formed from crystalline cellulose (Sigmacell Type 20) by both YEG1 and YEG2. The major product formed by YEG1 and YEG2 from all substrates was cellobiose with trace amounts of glucose. The K_m values for YEG1 and YEG2 against cellopentaose were 0.7 mM and 4.1 mM, respectively and against CMC, 1.83 mg/ml and 1.48 mg/ml, respectively. The K_m values of YEG2 on cellotetraose and cellotriose were 6.1 and 22.7 mM, respectively. Polyclonal mouse anti-serum was made against YEG2 using pure YEG2. It cross-reacted with YEG1 to the same extent as it did to YEG2, suggesting a common origin for both components. Western blotting and immunohistochemistry, using this anti-serum showed the presence of YEG1 and YEG2 in the salivary glands, but their absence from the midgut epithelium. It is proposed that the salivary glands are the major site of secretion of cellulase activity in *R. speratus*.

05-145

BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS OF *COPTOTERMES ACINACIFORMIS* (FROGGATT) IN LABORATORY AND FIELD COLONIESN. Lo¹, M. Slaytor¹ and R. H. Eldridge²¹Department of Biochemistry, The University of Sydney, Sydney, Australia - ²State Forests of NSW Research Division, West Pennant Hills, N.S.W., Australia

Biochemical and physiological parameters of *C. acinaciformis* were measured to test the validity of using termites maintained in an accelerated field simulator (AFS) rather than termites in the field for wood preservation studies. It is known that termites brought from disrupted field colonies into the laboratory undergo rapid changes in nitrogen metabolism. The reasons for these changes are not understood, but may be related to the disruption of the social structure when the nest is destroyed.

In the AFS, an intact nest of *C. acinaciformis* from the centre of a tree trunk was placed in a nest tank connected to a feeding tank and a soil-based foraging area. For comparative purposes, termites were collected as needed from nearby field colonies. There were no significant differences between the two groups of termites in respiratory activity, cellulase activity, uric acid content, total nitrogen content or nitrogen fixing ability. In contrast, termites taken from the field or the AFS and stored in nest material in the laboratory show a characteristic decline in nitrogen fixation levels as well as an increase in uric acid storage. These data suggest that termites are metabolically unchanged when maintained in the AFS and hence can justifiably be used in assessing wood susceptibility to termite attack.

05-146

STAGE SPECIFIC EXPRESSION OF FIBROINASE ACTIVITY IN THE SILK GLAND OF THE SILKWORM, *BOMBYX MORI*

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Fibroinase which hydrolyzes liquid fibroin and sericin was purified from the supernatant fraction of silk gland homogenate at stage D2 of the fourth molt larvae in the silkworm, *Bombyx mori*. It is a cysteine proteinase similar in kinetic properties to cathepsin L with a subunit molecular weight of 34kDa. It hydrolyzes liquid fibroin like an endopeptidase. Based on this catalytic property, an assay method of fibroinase using liquid fibroin as a natural substrate was refined and a tentative enzyme unit was defined. Developmental changes in fibroinase activity in the silk gland were determined by the modified assay method. A synthetic substrate for cathepsin L activity was also used. Two activity peaks in terms of g wet weight of tissue were observed, the one at stage D2 of the fourth molt larvae and another at the time of end of spinning. A similar profile of activity was observed using a synthetic substrate for cathepsin L activity. Fibroinase is conceivable to be a new molecular marker for the study of stage specific function of silk gland tissue. Since the silk gland does not degenerate in the fourth molt larvae but is programmed to degenerate in the early pupae in the presence of fibroinase activity in the tissue at both developmental stages, by differential screening of other tissue proteinases using fibroinase as a reference proteinase, mechanisms of tissue degeneration in the silk gland is highly likely to be elucidated.

05-148

PRELIMINARY CHARACTERIZATION OF AN ENTOMOTOXIC PROTEIN PRODUCED *IN VITRO* BY THE *STEINERNEMA CARPOCAPSAE* - *XENORHABDUS NEMATOPHILUS* COMPLEX.

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An entomotoxic protein was obtained by the incubation *in vitro* of the *Steinernema carpocapsae* - *Xenorhabdus nematophilus* complex. A partial characterization of the entomotoxic fractions proved them to be thermolabile protein, with activity above pH 5 and under pH 10. The entomotoxic protein was partially purified until a specific activity of 3795AT/mg protein, with a purification factor of 113 and a yield of 2%. In reducing PAGE/SDS, the electrophoretic analysis of the entomotoxic fraction revealed several protein bands and the relative molecular weight of a band supposed to be the responsible for the entomotoxic activity was estimated to be approximately 43 KDa. The hemocoelic injection into *Galleria mellonella* larvae of the toxic aliquots caused the paralysis of the treated larvae, followed by tetanization and death within 24 hours.

05-147

RIBOSOMAL PROTEIN L5 cDNA OF *BOMBYX MORI*: SEQUENCE AND TRANSCRIPTION

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Ribosomal protein L5 is associated with eukaryotic 5S rRNA, forming a precursor complex of the 60S ribosome subunit. The sequence of L5 has been established in molecular biology studies in yeast, rice, a nematode, and several vertebrates. We now report on isolation of a 1 kb cDNA encoding L5 of *Bombyx mori*. Nucleotides of the coding region and the deduced amino acids exhibit over 65% and 75% homologies with the respective sequences of the vertebrate L5 (nucleotides and amino acids shared by *Xenopus*, chicken, rat and man were considered), progressively lower homologies were found with the nematode, plant and yeast L5. *Bombyx* contains a single copy of the L5 gene that is constitutively expressed in all examined cell types but the level of expression seems to reflect the intensity of proteosynthesis. The highest amount of L5 mRNA occurs in the silk glands. It is virtually constant throughout the penultimate and last larval instars but drops sharply after the cocoon spinning. Interestingly, the degenerating pupal silk glands retain a small amount of the L5 transcript.

05-149

RESPONSES OF CHESTNUT MOTHS TO SEMIOCHEMICALS: ELECTROPHYSIOLOGY AND FIELD TESTS

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Electrophysiological responses of males and females *Pammene fasciana* (L.), *Cydia fagiglandana* (Zeller) and *C. splendana* (Hübner) on stimulation with tortricid sex attractants and with volatiles from chestnut leaves are reported. Field tests were carried out in different chestnut areas of Southern Italy.

Z8-12:Ac elicited the higher EAG response for *P. fasciana*. Traps loaded with this compound catch high number of *P. fasciana*; their attractant power and selectivity are improved adding Z8-12:OH.

EAG recordings showed that E8E10-12:Ac and its isomers (Z,E; E,Z; Z,Z) highly stimulated the antennae of *C. fagiglandana* and *C. splendana*. Dose-response curves were almost identical for males of both species. Females antennae were also stimulated and EAGs were up to 50 times lower. Field tests showed that traps baited with E8E10-12:Ac or Z8E10-12:Ac catch high and low number of males of *C. fagiglandana* and *C. splendana*, respectively. Mixtures of E8E10-12:Ac with E8E10-12:OH improved the trap selectivity to *C. fagiglandana*. *Cydia* species are also able to smell Z8-12:Ac. Traps loaded with mixtures of E8E10-12:Ac with Z8-12:Ac do not catch males of the three species.

Preliminary single-cell recordings (Den Otter *et al.*, *in press*) from *C. fagiglandana* males suggested that E8E10-12:Ac activated cells with medium-sized spikes, E8E10-12:OH and Z8-12:Ac these same cells and cells with large spikes and small spikes, respectively. Some chestnut volatiles activated cells with the medium-sized spikes only.

05-150

ELECTROANTENNOGRAM RESPONSES OF THE RICE BROWN PLANT HOPPER *NILAPARVATA LUGENS* TO PLANT VOLATILE COMPOUNDSY.N. Youn, L.J. Goodman¹

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Electroantennogram recordings were made from the plaque organ receptors of the rice brown plant hopper, *Nilaparvata lugens*. The receptors of the distal plaque organ of male and female hoppers responded to the air from above chopped rice plant although females gave a larger response. Female hoppers, tested with 27 plant volatiles, gave dose-related responses to 16 of the compounds, including a range of green leaf volatiles. The aliphatic aldehydes hexanal and *trans*-2-hexenal elicited a larger response than the green leaf alcohols tested. Acetophenone, benzaldehyde, ethyl and methyl benzoate and amyl and isoamyl acetate were among the most effective of the remaining compounds tested. Receptor recovery times for dose-response related compounds were generally quite similar with the exception of *trans*-2-hexenal which required exceptionally long recovery periods. A comparison of the dose-related response of 16 compounds made up in paraffin oils and pentane showed that receptors appeared considerably more sensitive to the compounds when the highly volatile solvent pentane was used. Both female and male hoppers responded to the air from above male hoppers. Male hoppers were sensitive to the air above rice leaf sheath containing hopper eggs.

05-151

INSECT PHYSIOLOGY SERVING SOCIETY IN THE 21st. CENTURY

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The current age of biology has a dominant place for insect physiology. This talk will present the historical context for this prediction, and provide examples of growth areas for insect physiology in the 21st. Century. The 20th. Century saw a tremendous increase in our knowledge about how insects function, but we are lacking depth and breadth in our understanding of insect life processes. However, this situation is changing rapidly with developments in instrumentation, computer technology, and molecular biology making insects increasingly amenable to physiological and biochemical study. Advances in insect biochemistry, genetics, and molecular biology are providing the foundation for technological innovations in the genetic engineering of plants resistant to insects and the development of new biorational control procedures. Advances in physiological ecology in such areas as the mass rearing of natural enemies, molecular systematics, and seasonal development and behavior are providing the foundation for biologically intensive integrated pest management as part of holistic resource management. Furthermore, biologists are realizing the potential of insects as model animal species for physiological and biochemical studies because of their ease of rearing in large numbers, relatively low maintenance costs, and short life cycles.

05-152

Juvenile hormone metabolic activity in fat body from *Pseudoplusia includens* injected with *Microplitis demolitor* calyx fluid.

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Calyx fluid from the braconid parasitoid *Microplitis demolitor* suppresses host hemolymph JH esterase activity when injected into last-stadium *Pseudoplusia includens*. Evidence suggests that the effect of *M. demolitor* calyx fluid on host JH esterase activity is not due to a humoral factor. We will measure JH esterase levels and JH esterase and epoxidase activity in tissues from control and calyx fluid-injected *P. includens*.

05-153

ANALYSIS OF PARASITISM SPECIFIC PROTEINS IN THE PARASITOID-HOST SYSTEM *CHELONUS INANITUS*-*SPODOPTERA LITTORALIS*

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Parasitism by the egg-larval parasitoid *C. inanitus* induces in its host *S. littoralis* the precocious onset of metamorphosis in the 5th stadium while nonparasitized larvae pass through 6 stadia (Grossniklaus et al., 1994, *Invert. Reprod. Develop.* 25, 143-158). We could recently show that the parasitoid larva plays a key role in inducing precocious metamorphosis (Pfister-Wilhelm and Lanzrein, *Arch. Insect Biochem. Physiol.*, in press). In order to investigate a potential role of parasitoid-made proteins in this process, we produced antibodies against proteins released by parasitoid larvae in vitro. With these antibodies we analyzed the presence and persistence of parasitoid-made proteins in haemolymph of host larvae. A protein of approx. 200 kD was observable from the 3rd larval instar onwards and three others appeared in the transition from the 4th to the 5th larval instar. By parasitoid removal experiments the persistence of these proteins was investigated and correlated with the developmental fate of such parasitoid-deprived host larvae. We also collected and concentrated medium in which parasitoids had been cultured and injected it to study the effect on development and metamorphosis of recipient larvae.

05-154

POLYDNAVIRUS DNA OF THE BRACONID WASP *CHELONUS INANITUS* IS INTEGRATED INTO THE WASP GENOME AND EXCISED IN THE FEMALES AT LATE STAGES OF PUPAL-ADULT DEVELOPMENT

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The polydnavirus (PDV) of the braconid wasp *C. inanitus* has been characterized and the genome was shown to consist of double stranded circular DNA molecules of sizes between 7-31 kbp (Albrecht et al., 1994, J. Gen. Virol. 75, p. 3353-3363). We have investigated whether viral DNA is integrated in the wasp genome as shown for the ichneumonid wasp *Campoletis sonorensis* (Fleming and Summers 1991, Proc. Nat. Acad. Sci. USA, 88, p. 9770-9774). We screened a *C. inanitus* adult male genomic library with a fragment of a 12 kbp viral segment and obtained a positive clone in which viral and flanking genomic sequences were analyzed. This is the first direct demonstration of integration of PDV DNA in a braconid wasp. By the use of PCR primers which distinguish between the integrated and the excised and circularized form of viral DNA, we show that the integrated form is present in both sexes at all developmental stages and excision and circularization of viral DNA begins in females at late stages of pupal-adult development.

05-156

ENDOCRINE CHANGES ASSOCIATED WITH THE POLYDNAVIRUS/VENOM INDUCED DEVELOPMENTAL ARREST IN THE PARASITOID-HOST SYSTEM *CHELONUS INANITUS*-*SPODOPTERA LITTORALIS*

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The presence of polydnavirus (PDV) and venom of the egg-larval parasitoid *C. inanitus* has been shown to induce developmental arrest in the prepupal stage of *S. littoralis* (Soller and Lanzrein, J. Insect Physiol., in press). We have compared various endocrine parameters between nonparasitized last instar larvae and last instar larvae parasitized by X-ray irradiated wasps. Such wasps oviposit normally into *S. littoralis* eggs and inject PDV and venom. However, the parasitoid eggs are infertile and no parasitoid develops. In X-ray parasitized larvae we observed, as compared to nonparasitized larvae, a slight alteration of juvenile hormone (JH) titres in the early feeding stage, a delay and depression in the post-wandering JH esterase and delayed and desynchronized increases in prepupal JHs and ecdysteroids. Only minor differences in ecdysone metabolism were observed, and in vitro incubation of prothoracic glands indicates that the biosynthesis of ecdysone is suppressed in X-ray parasitized larvae at the cell formation stage.

05-155

INTERFERENCE OF THE PARASITOID *CHELONUS INANITUS* WITH THE JUVENILE HORMONE SYSTEM OF THE HOST *SPODOPTERA LITTORALIS*

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The egg-larval parasitoid *C. inanitus* (Braconidae) induces in its host *Spodoptera littoralis* (Noctuidae) the precocious onset of metamorphosis, i.e. parasitized larvae enter metamorphosis in the 5th stadium and non-parasitized larvae do so in the 6th stadium. We could recently show by parasitoid removal and transplantation experiments that the parasitoid larva plays a key role in manipulating host metamorphosis (Pfister-Wilhelm and Lanzrein, Arch. Insect Biochem. Physiol. in press). We show here data on effects of parasitoid implantation on the *c. allata* activity and on the juvenile hormone (JH) titres of recipient larvae and correlate these with the developmental fate of such larvae.

05-157

HOST REGULATION EFFECTS OF OVARY EXTRACTS AND VENOM OF *APHIDIUS ERVI* HALIDAY (HYMENOPTERA, BRACONIDAE)

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The aphid parasitoid *Aphidius ervi* Haliday regulates development, metabolism and reproduction of its host *Acyrtosiphon pisum* (Harris) (Pennacchio et al., 1995, Arch. Insect Biochem. Physiol., 30, 351-367). The ovary development is strongly affected by the parasitoid. Parasitism of pea aphid 1st instars results in a complete inhibition of ovary development associated to a subsequent developmental arrest of the host, which is unable to reach the adult stage and dies as 4th instar. Arrested hosts show a reduced titer of total ecdysteroids, compared to synchronous nonparasitized controls. These parasitoid-induced alterations were in part duplicated by injecting into *A. pisum* 4th instars nonphysiological doses of venom gland and ovary extracts from *A. ervi*. The most evident symptom after injection was the arrest of the development and death as 4th instar, while saline injected controls usually attained the adult stage and reproduced. Electron microscopy observations did not show the presence of viral particles both in the ovary and venom gland. The highest rate of developmentally arrested aphids was obtained by injecting a mixture of ovary and venom gland extracts (1:1), while separate injections of these two extracts were significantly less effective and induced similar results. Heat treatment and protease digestion of the combined extracts both eliminated host developmental arrest, suggesting that a protein could be the host regulatory factor involved. SDS-PAGE analysis of ovary and venom extracts showed that they have a similar electrophoretic pattern. Further studies are in progress to isolate the active component/s.

05-158

DEVELOPMENTAL EFFECTS AND EXPRESSION OF POLYDNA VIRUS GENES FROM *MICROPLITIS CROCEIPES* (CRESSON) IN PERMISSIVE AND NON-PERMISSIVE HOSTS (HYMENOPTERA: BRACONIDAE - LEPIDOPTERA: NOCTUIDAE AND PYRALIDAE)

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In the natural host parasitoid association, *Helicoverpa zea* (Boddie) and *Microplitis croceipes* (Cresson), and in association with a semi-permissive host *Galleria mellonella* (L.), injections of calyx fluid and venom differentially affected growth and development of the hosts, however in a non-permissive host, *Spodoptera exigua* (Hübner), injections of calyx fluid and venom had a minimal effect. Expression of polydnavirus genes from *M. croceipes* in the three host species is being examined.

05-160

INFLUENCE OF PARASITIZATION BY THE SOLITARY PARASITOID *GLYPTAPANTELES PORTHETRIAE* ON THE DEVELOPMENT AND JUVENILE HORMONE DEGRADATION OF ITS HOST *LYMANTRIA DISPAR* (HYMENOPTERA: BRACONIDAE - LEPIDOPTERA: LYMANTRIIDAE)

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Although the solitary endoparasitic braconid *Glyptapanteles porthetriae* (Muesebeck) is an abundant, natural controlling agent of the gypsy moth throughout its native range only little is known about its development.

Under natural conditions *G. porthetriae* attacks mainly first instar gypsy moth larvae. Fresh weight of the parasitized larvae in the third instar is significantly lower than the fresh weight of unparasitized control larvae of the same stage. During the late third instar the host stops feeding and the parasitoid emerges several days later. By that time control larvae have molted into the fourth instar. Larval development and molting is depending on the juvenile hormone titer (JH) in the hemolymph of the host. JH in the blood of gypsy moth was shown to be degraded by only one enzyme, the juvenile hormone esterase (JHE). Two peaks of JHE activity occurred in third instar control larvae, one at the beginning and a second one at the end of the instar. In the hemolymph of parasitized larvae only the first peak was found. Later in the third instar hardly any JHE activity was detected. This blocking of the JHE activity by the parasitoid might be the reason for a high JH titer in the host's hemolymph which subsequently causes disruption of development. Premature death of the host results in a significant decrease in feeding damage compared to the damage caused on oak by normally developing gypsy moth larvae.

05-159

CHANGES IN JUVENILE HORMONE ESTERASE ACTIVITY AND ECDYSTEROID TITER IN LARVAE OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA*, PARASITIZED BY THE WASP *ENYTUS MONTANUS*.

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Last-instar *Choristoneura fumiferana* larvae parasitized by *Enytus montanus* early in the stadium display retarded development and fail to initiate metamorphosis. Similarly, injection of the wasp's calyx fluid (CF) into healthy caterpillars induces a dose-dependent delay in pupation, which can be abolished by prior treatment of CF with UV and the DNA cross-linker psoralen. These results suggest that the wasp's polydnavirus is the factor responsible for the observed developmental disruptions.

In an effort to identify the physiological processes that are perturbed by *E. montanus* CF, we examined the effects of parasitism and injection of CF on host plasma juvenile hormone esterase (JHE) activity and ecdysteroid titers in last-instar budworms. Under our experimental conditions, control larvae displayed only one peak of JHE activity (~ 30 nmol JH III/min/ml), which occurred on day 4 of the last stadium. In saline-injected animals, a similar peak was observed, but on day 5. In larvae parasitized or injected with CF (0.5 female equivalent) on day 1, levels of JHE activity were depressed during the entire stadium, with a somewhat greater reduction induced by CF injection (~ 3 nmol JH III/min/ml on day 4) than parasitism (~ 8 nmol JH III/min/ml on day 4). Similarly, parasitism and injection of CF both completely prevented the rise in hemolymph ecdysteroid titer observed in control and saline-injected larvae between day 3 and day 7 (rises from ~ 25 to 500-700 pg 20-hydroxyecdysone equivalents/μl hemolymph). We are currently examining the effect of *E. montanus* CF on rates of host JH biosynthesis and prothoracic gland integrity as well as the effect of « Growth Blocking Peptide » (1995; J. Insect Physiol. 41: 1) on the same variables.

05-161

DOWN-REGULATION OF FECUNDITY IN FEMALE *TENEbrio MOLITOR* (COLEOPTERA) IS INDUCED BY HAEMOLYMPH FROM *HYMENOLEPIS DIMINUTA* (CESTODA) INFECTED BEETLES.

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Fecundity reduction, which occurs in *H. diminuta*-infected *T. molitor*, is associated with changes in the hormonal control of vitellogenesis. Reduction of binding of juvenile hormone to the host follicular epithelium may be due to the presence of a modulatory molecule(s) of either parasite or host origin.

To investigate the hypothesis that such a molecule is present in the haemolymph of *H. diminuta*-infected *T. molitor*, haemolymph from both infected female and male beetles (and controls) was injected into non-infected recipients. To assess any subsequent fecundity reduction, levels of protein in individual ovarian follicles were monitored by ELISA using polyclonal antibodies raised against *T. molitor* vitellin.

Infected beetles harboured parasites at three different developmental stages, 1, 3 to early stage 4 and the mature stage which is infective to rats. Only haemolymph from infected females with parasites at stages 1 and 3 to early stage 4, elicited fecundity reduction in non-infected recipients. Our results thus indicate the presence of fecundity modulatory molecules which are circulating in the haemolymph of *H. diminuta*-infected *T. molitor*.

05-162

GLUTATHIONE S-TRANSFERASES FROM THE FRUITFLY *BACTEROCERA PAPAYAE*

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Glutathione S-transferases (GSTs) belong to a group of enzymes implicated in the detoxification of xenobiotics including chemical insecticides. We are studying the GSTs in *Bacterocera* in order to understand the biochemical mechanisms involved in insecticide resistance.

Cytosol prepared from *B. papayae* larvae showed activity towards 1-chloro-2,4-dinitrobenzene (CDNB), a universal substrate for GSTs. In addition, 1,2-dichloro-6-nitrobenzene (DCNB) activity was also detected in the cytosol. The specific activities of the *Bacterocera* GSTs were comparable to GSTs purified from other insecticide resistant species.

Purification of GSTs were carried out using glutathione sepharose 4B affinity chromatography. The purified enzymes were used to generate polyclonal antibodies which will aid in the study of the expression and regulation of GSTs in this insect.

05-164

LIPID MOBILIZATION IN ADULT *Manduca sexta*: PATHWAY FOR THE SYNTHESIS OF SN-1,2-DG.

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The pathway for the synthesis of sn-1,2-DG induced by adipokinetic hormone (AKH) was examined. The precursor-product relationship between possible intermediaries was measured after lipid mobilization was induced by AKH. In order to radiolabel the fat body triacylglycerol (TG) pools, late fifth instar larva were fed either [9,10(n)-³H]oleic acid, [9,10-³H]palmitic acid or [1(3)]-³Hglycerol, respectively. This procedure yielded adult insects in which the radioactivity found in the fat body TG represented about 85 % of total radioactivity present in fat body and hemolymph. The positional distribution of radiolabeled fatty acid in the TG showed that 83 % of the label was incorporated in the sn-2-position. Insects fed radiolabeled glycerol showed 80% of the radioactivity in the glycerol backbone and 20 % in the fatty acids. Fat bodies were taken out 1-60 min after AKH injection and total lipid extracts were prepared. The analysis of the radiolabeled lipid components of the fat bodies showed that neither monoacylglycerol nor phosphatidic acid were presents. Conversely, sn-1,2-DG was the only component of the fat body that had a significant increase by the action of AKH. The onset of the DG increase began about 10 min after AKH injection. This result correlated well with the activation of the fat body TG-lipase. The lipase activation began around 5 min after AKH injection and was maximal at 10 min. The release of DG into the hemolymph began about 10 min after AKH injection followed by a gradual increase of the lipid level and the maximal response was observed 2 h after the injection. These results indicated that the stored TG is the precursor of the sn-1,2-DG. The stereospecific hydrolysis of TG by the TG-lipase originates the DGs which are released from the fat body into the hemolymph. This work was supported by NIH grant GM 51296.

05-163

SIZE, COMPOSITION AND PROPERTIES OF THE LIPOPHORIN SURFACE LIPID DOMAIN.

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The transformation of HDLp into IDLp and LDLp is the result of the progressive loading of HDLp with DG and an exchangeable apolipoprotein, apolipoprotein-III (apoLp-III). The increase in apoLp-III content, from 0 to 16 molecules per particle, is accompanied by a gradual increase in the zeta-potential which, at pH 8.6, ranges from +1.02 mV for lipophorins without apoLp-III to -7.76 mV for lipophorins containing 16 molecules of apoLp-III. As judged by the changes in the partition constant for trimethylammonium diphenylhexatriene and oleic acid, an average 2-fold increase in the size of the lipophorin lipid-surface takes place when HDLp is loaded with DG and transformed in LDLp. These data, as well as the results obtained by end-point lipolysis with a triacylglycerol (TG)-lipase, indicated that the accessible DG-content increases four to seven times when HDLp is converted in LDLp. Fluorescence polarization of the cationic and anionic lipid probes, trimethyl ammonium diphenylhexatriene and cis-parinaric acid, embedded in eight different subspecies of lipophorin, containing from 12 to 50% DG, showed a small decrease in the surface lipid order when going from HDLp (25% DG) to LDLp (50% DG). Porcine pancreatic phospholipase A₂ was used as probe of the lipoprotein surface. As the DG-content of the lipoprotein increased, a higher enzyme activity against the lipoprotein-phospholipids was observed, with a maximum activity five-fold higher against LDLp than against HDLp. The changes in charge, PLA₂ and triacylglycerol-lipase activity, lipid order and size of the lipophorin lipid-surface provide a link between the structure and properties of the lipophorin surface and the physiological roles of HDLp and LDLp particles. Supported by NIH grant GM50008.

05-165

A TRIACYLGLYCEROL-RICH LIPOPHORIN IN THE YELLOW FEVER MOSQUITO, *Aedes aegypti*

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Lipophorin, the major lipid transport vehicle in insect hemolymph, was isolated from the yellow fever mosquito *Aedes aegypti*. This lipophorin had all the characteristics of the typical insect lipophorin, with the exception that its major neutral lipid component was triacylglycerol (TG) versus diacylglycerol (DG) in other insect species. Insect lipophorin is known for its ability to function as a reusable shuttle in the transport of lipid in hemolymph. The presence of TG may have implications on the metabolism of lipophorin. We investigated whether *A. aegypti* lipophorin functions as a lipid shuttle or whether its metabolism is more similar to that of vertebrate lipoproteins.

Compared to the DG-rich lipophorin of the sphinx moth *Manduca sexta*, *A. aegypti* lipophorin loaded neutral lipid from fat body much less efficiently. Neither concentration, nor density of lipophorin in adult female mosquitoes was affected by starvation, when compared to sugar feeding. Ingestion of a blood meal, however, resulted in a three-fold increase in lipophorin level and a slight increase in lipophorin density, both at 40 h post feeding. These results indicate that *A. aegypti* responds to a need for increased lipid transport by synthesizing and secreting more lipophorin into the hemolymph, rather than by loading more lipid onto existing lipophorin.

05-166

CHARACTERIZATION OF THE LIPOPHORIN RECEPTOR FROM THE MIDGUT OF *Manduca sexta*.K. C. Gondim and M. A. Wells

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In insects lipids are transported in the hemolymph by lipophorin. Lipophorin acts as a reusable shuttle for lipids, that is, it transfers lipids to the sites of storage or utilization, but the apoproteins of the particle are not accumulated in these tissues, and the lipoprotein can be reloaded with more lipids and reutilized. In the feeding larvae of *Manduca sexta*, lipophorin receives lipids from the midgut and transports them to the fat body, where they are stored. In order to understand how lipid transport in these insects is regulated, we are characterizing the lipophorin receptor from the larval midgut. Midguts were dissected from feeding fifth instar larvae and a membrane preparation was obtained. A filtration assay was used to measure the binding of purified iodinated lipophorin (^{125}I -lipophorin) to its receptor. Non-specific binding was determined in the presence of an excess of non radioactive lipophorin, and the specific binding by the difference between the total and non-specific binding. A time-course of lipophorin binding to the membranes showed that it is a very rapid process, and that equilibrium was reached within 40 min. In the presence of increasing concentrations of membrane protein, corresponding increases in lipophorin binding were observed. Preliminary results indicated that the binding of lipophorin to the midgut receptor does not depend on the presence of calcium and that it can be inhibited by suramin, in a dose dependent manner. Lipophorin binding decreased with increasing pH values, in the range between 5.5 and 8.5. When the membranes were incubated with increasing concentrations of ^{125}I -lipophorin, a saturation curve was obtained, with a K_d in the range of 40 $\mu\text{g/ml}$. It is possible that lipophorin containing different amounts of lipids have varying affinities for the receptor, and this will now be investigated. Supported by: NIH Grant GM 50008

05-167

THE STRUCTURAL APOPROTEINS OF LIPOPHORIN, APOLIPOPHORIN-I AND II, ARE ENCODED BY A SINGLE mRNA: (DNA AND DEDUCED AMINO ACID SEQUENCE)K. A. Sundermeyer and M.A. Wells

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Lipophorin is the major hemolymph lipoprotein in insects. It is a large particle that varies in size from 600 to 1000kD, depending on the amount of lipid it contains. Each lipophorin particle consists of two structural apoproteins: apolipophorin I (apoI), $\text{Mr} \approx 250\text{kD}$, and apolipophorin II (apoII), $\text{Mr} \approx 80\text{kD}$. The amino acid sequence of apoI and apoII will help to elucidate many aspects of the structure, function, and biosynthesis of these apoproteins. ApoI and apoII are transcribed into a single 10.5kb mRNA, translated into a single, large polypeptide ($\text{Mr} \approx 350\text{kD}$), and post-translationally cut into apoI and apoII. In this poster we analyze and discuss the main features of the cDNA and the derived amino acid sequence of apoI and apoII from *Manduca sexta* fat body. We also describe the methods and problems encountered in cloning and sequencing a large cDNA. Supported by NIH grant GM50008

05-168

CHARACTERISTICS AND SYNTHESIS OF APOLP-III IN *SPODOPTERA LITURA*Eung-Seok Kim, Beom-Su Kim, So-Joung Choi, Hak R. Kim

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ApoLp-III was separated and then purified from the hemolymph of last instar larvae of *Spodoptera litura*. Characteristics and synthetic place were investigated during development. ApoLp-III has molecular weight of 18.6 kDa and contains 17.3% aspartic acid and 13.9% alanine but tryptophan was not detected. Based on N-terminal sequence, apoLp-III of *S. litura* has 48.6% homology with *Manduca sexta* and 30% homology with *Hypantria cunea* but only 11% with *Locusta migratoria*. Fat body, ovary, and testis were tissue-cultured during development. ApoLp-III was synthesized in fat body with great activity in adult stage and also in ovary during early developmental stage. ApoLp-III was confirmed to be present in testis and ovary throughout whole developmental stage by SDS-PAGE. Especially location of apoLp-III in testis was also investigated.

Section 6

Insect Neurosciences

06-001

ANCIENT STRUCTURES IN MODERN BRAINS: INSECT PHYLOGENY FROM THE NERVOUS SYSTEM.

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Attempts to trace the evolutionary history of animals have traditionally relied on features that are sustained through geological time, such as an exoskeleton that is at least in part preserved in the fossil record and can be used for comparative morphology. More recently, comparison of conserved DNA and RNA have providing insights into relatedness at a variety of taxonomic levels. Here I shall report on novel efforts to construct phylogenies from parsimony analysis using criteria derived from central neural characters.

Results from phylogenetic analysis based on over 60 independent neural characters amongst 23 major arthropod taxa is convergent with results by others obtained from molecular studies. Cladistic analysis demonstrates the ancient origin of certain neural structures, such as the mushroom bodies, which are found throughout the arthropods and annelids. The same technique at a finer level of resolution now suggests that, amongst the Insecta, the Holometabola are polyphyletic and that their constituent taxa have been shoe-horned into this assemblage. Evidence from conserved structures in the phylogenetically ancient olfactory centers and mushroom bodies suggest that the Hymenoptera may be closer to the Orthoptera than to other orders ascribed to the Holometabola. Studies of the central body complex, an insect "invention," supports this view.

06-002

IS VENTRAL IN INSECTS DORSAL IN VERTEBRATES?

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The ventral, respectively dorsal nerve cords of insects and vertebrates are generally thought to have evolved independently. Based on molecular and embryological evidence we have recently proposed that the ventral side of insects corresponds to the dorsal side in vertebrates, and that a gastroneuralian ancestor - during its evolution into a chordate - has inverted its dorsoventral body axis. If so, then the ventral, respectively dorsal nerve cords of insects and vertebrates would be homologous structures.

We have now compared the early development of insect and vertebrate brain anlagen. Based on published data we discuss that in the anlagen of insect and vertebrate brains and nerve cords similar genes are being expressed in comparable brain areas with similar functions in the adult. In addition, early axogenesis is also very similar. Insect and vertebrate brains thus appear to develop according to a common ground plan that may have existed already in their common ancestor. - It is proposed that enteropneusts may serve as a unifying conceptual link between gastroneuralian invertebrates and notoneuralian chordates.

The idea that arthropods and vertebrates are built according to a common body plan (with arthropods having chosen to walk around „upside down“) has been proposed some 150 years ago by Geoffroy St.-Hilaire. According to Dohrn (1875), however, vertebrates derive - by dorsoventral inversion - from an annelid-like ancestor with a ventral nerve cord. We show that molecular genetics supports the idea that insects and vertebrates derive from a common gastroneuralian ancestor, and that chordates have inverted their dorsoventral body axis to bear their nerve cord henceforth on their dorsal side.

06-003

EMBRYOGENESIS OF THE INSECT BRAIN: FORMATION OF PROLIFERATIVE CLUSTERS, CELL TRANSLOCATION, AND CORTICAL ORGANIZATION

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During embryogenesis in the grasshopper, a developmental program transforms a relatively simple, two-dimensional sheet of neuronal precursor cells - called neuroblasts - into the highly complex structure of the mature brain. The transformation begins with the aggregation of the neuroblasts into at least 5 discrete proliferative clusters: pars intercerebralis, protocerebrum, deutocerebrum, tritocerebrum and midline. Aggregation into a cluster is the result of a progressive wrapping of neuroblasts and their progeny by glia cells. These brain-specific proliferative aggregates are established well before axogenesis begins. Since the initial pioneer neurons navigate between the glial-bound proliferative clusters to establish the primary axon scaffold, the clusters may prefigure neuropilar pathways in the brain.

Following establishment of the proliferative clusters, a morphogenesis occurs in which the clusters of proliferating neuroblasts behave as mobile plate-like structures that become repositioned relative to one another in the head. Further, individual identified neuroblasts are displaced independently of other neuroblasts within their proliferative cluster. Translocation may follow routes laid down in the early pre-axogenesis by glia cells. Displacement of putatively equivalent neuroblasts to identical cortical locations in the brain of the grasshopper, the stick insect and the cockroach suggests that the same developmental program operates in various orthopteroid insects.

06-004

THE "CENTRAL COMPLEX" IN THE ARTHROPOD BRAIN: IMMUNOREACTIVITY TO PEPTIDE ANTISERA IN SPIDERS AND INSECTS

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Arthropods are usually considered a monophyletic group, and a central brain structure, the "central body", has been recognized in all arthropod groups (Holmgren, 1916). Based on differences in the position, connectivity, and development of the central body, however, Strausfeld (1993) and Breidbach (1995) suggested that the central bodies of arachnids and insects are not homologous.

We applied immunocytochemical methods to investigate parallels and differences in the neuronal architecture of the central body in insect and spider brains. Our study revealed that antibodies to the neuropeptides allatostatin 1, proctolin, and crustacean cardioactive peptide (CCAP) label neurons of the "central body" in the spider *Cupiennius salei* (Ctenidae) as well as in insects (*Schistocerca gregaria*, *Periplaneta americana*).

In insects the central body together with the protocerebral bridge are subcompartments of the central complex, while a distinct protocerebral bridge has not been recognized in the brain of arachnids. In arachnids and insects, the central body/central complex is a highly ordered neuropil extending across the midline of the brain. In both groups the structure consists of horizontal layers and transverse columns (16 columns in insects, numerous columns in spiders), which in insects form regular fiber chiasmata between the bridge and the central body. In the spider as well as in the locust and cockroach, the central body/central complex is closely connected to the visual system, in part by CCAP-immunoreactive fibers. Allatostatin-immunostaining revealed columnar and tangential elements in both arthropod groups. Detailed morphological analysis suggests interactions of columnar elements from the two brain hemispheres in the central body/central complex.

In summary, peptide immunostaining of the central complex of insects and the central body of spiders reveals remarkable similarities in internal organization and connections to the visual system which do not support the hypothesis that these structures have evolved twice in chelicerates and in insects.

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06-005

THE CENTRAL COMPLEX IS A HIGHER CENTRE FOR LOCOMOTOR CONTROL IN INSECTS. EVIDENCE FROM NEW *DROSOPHILA MELANOGASTER* MUTANTS (DIPTERA: DROSOPHILIDAE)

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Cladistics suggest that the central complex of the brain (CC) might be special to insects and to their evolution of locomotion and orientation. The behavioural significance of the CC for walking and certain aspects of orientation was deduced from comparative behavioural studies in *Drosophila* mutant lines with known structural disorders in the CC (Strauss & Heisenberg 1993; J Neurosci 13:1852-61). In the present study we isolated 230 lines out of 10,750 mutagenized flies for their walking impairments in a behavioural screen (Strauss; J Neurogenet, in press). The coordination of legs, joint angles, step lengths, and duration of swing phases were analysed in high resolution using a computerized detector system (Strauss 1993; J Neurogenet 8:250). Their external body and gross brain morphology were inspected.

In 30 lines walking impairments were correlated with structural defects in the CC detectable with light microscopy. Considering the neuronal and non-neuronal extent of the walking system this unexpectedly large fraction of 13% of all lines emphasizes its importance as a higher centre for locomotor control. Two exemplary new lines shall be considered here.

In mutant flies of the line *C141*, affected in an apparently thus far unknown gene, the protocerebral bridge of the CC is disrupted medially. Severe walking impairments, even when studied in high resolution, perfectly parallel those of *no-bridge*^{KS49} flies. The latter mutation in an independent gene causes a median gap in the protocerebral bridge as well (Strauss et al. 1992; J Neurogenet 8: 125-55). This finding strengthens the notion that the protocerebral bridge is involved in the adjustment of step lengths both to achieve turning and secondly to optimize walking speed during straight walking.

Flies of our line *C31* are impaired in the CC and by the same mutation in the thoracal ganglion. When the defective gene is expressed unilaterally throughout the fly, the mosaic individuals continuously turn to the mutated side even when approaching a landmark. The tendency to turn is not observed in unilaterally thorax-defective mosaics with intact brains nor in completely defective *C31* flies. The finding suggests that an intact CC is capable of compensating for the different performances of a defective and a normal body side. A *C31*-impaired brain, however, can no longer balance existing differences.

06-007

EVOLUTION OF THE MOTH EAR: COMPARATIVE PHYLOGENY AND ONTOGENY

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The moth ear represents a valuable opportunity to study the evolutionary pathways of an insect sensory system, both at the peripheral and at the central nervous system level. Moth ears have arisen polyphyletically from pre-adapted proprioceptors located at wing bases. The existence of earless taxa allows for the identification of the ancestral condition of the auditory chordotonal organ, a three-celled stretch receptor complex. This plesiomorphic condition has lost one cell to become the auditory organ in the Noctuidae and two cells in the Notodontidae. Why cellular loss has occurred in these taxa but not in the four-celled ears of the Geometroidea is discussed.

The larvae of Lepidoptera do not possess tympanal ears but have a chordotonal precursor located in the anatomically homologous location as the adult ear. Experiments that ablate the proprioceptor organ in last instar larvae result in adult ears with disrupted tympanal membranes lacking the auditory organ. These results suggest that metamorphosis, with its massive tissue reorganisation may have been the stage in which random mutations leading to the evolution of an ear took place.

06-006

EVOLUTIONARY CONSERVATION OF A PREDATOR EVASION SYSTEM.

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Predation has been a fact of life since pre-Cambrian times. The interplay of prey detection and predator evasion has accordingly been a major factor in the evolution of animal form and function. Among the several early-warning sensory systems that serve to alert potential prey, mechanoreceptor input appears to be the most ancient. It served the first terrestrial animals, the arthropods, and is retained to this day by many of them, including hexapods.

Another widespread component of predator evasion systems is the giant interneuron. Sets of interneurons with relatively large axonal diameters occur in many arthropods. Within the Hexapoda a set of abdominal giant interneurons that serve to convey mechanosensory input from abdominal cerci and adjacent mechanoreceptors to thoracic ganglia and the brain in the Archaeognatha, Thysanura and orthopteroid orders appear to be homologous. This apparently conserved system did not persist in recognisable form into the Holometabola, where visually driven descending giant interneurons are widespread.

06-008

CELL LINEAGE AND CELL FATE SPECIFICATION IN THE EMBRYONIC CENTRAL NERVOUS SYSTEM OF *DROSOPHILA*

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In the *Drosophila* embryo the CNS develops from a population of neural stem cells (neuroblasts, NBs) and midline progenitor cells which delaminate from the ectoderm according to a stereotyped spatial and temporal pattern. By labelling individual early gastrula cells with DiI we have clarified the entire embryonic lineages of the mesectodermal midline progenitors and of most of the thoracic and abdominal NBs. Some of the NBs give rise to segment specific lineages. The identified wildtype CNS lineages provide a foundation for the analysis of mutants, gene expression patterns and experimental manipulations.

Positional information plays an important role in the specification of the various neural progenitors. Heterotopic single cell transplantations reveal differential commitment of progenitors along the dorsoventral axis of the early gastrula neuroectoderm. These experiments and mutant analysis suggest that signals exist at ventral sites conferring ventral specificities to the cells, and that the ventral midline is involved in their correct positioning. This resembles the situation in vertebrates in which the identity of ventral cells and the position that they occupy in the neural tube appears to depend on signals from the ventral midline (floor plate). Ongoing experiments are aimed at elucidating the putative role of the *Drosophila* ventral midline in specifying cell fates in the adjacent neural and mesodermal tissues.

06-010

TRANSCRIPTION FACTORS IN THE DEVELOPING INSECT NERVOUS SYSTEM-APPROACHES TO THEIR FUNCTION AND THEIR USEFULNESS AS MARKERS

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The best characterised insect transcription factors are those involved in controlling segmentation and in determining segmental identity in embryonic *Drosophila*. These same proteins occur in predictable, and frequently serially homologous, subsets of neurons, later in development. A few functional studies have now been carried out using genetic techniques (*Drosophila*), or the injection of anti-sense oligonucleotides (grasshopper), to knock out specific transcription factors, but such studies are just beginning. Genes coding for homologs of the most studied *Drosophila* transcription factors have now been cloned from several other insects, most notably *Tribolium*, *Schistocerca*, *Locusta*, *Precis*, *Manduca*, and *Bombyx*. This allows interspecific comparison of individual transcription factors and a search for patterns of distribution that might suggest a function. For example, the co-occurrence of specific transcription factors in motoneurons and the muscles which they innervate raises the possibility that the factor is controlling expression of a common surface recognition molecule in the axon and its target. Clues to function may also come from changes in the pattern of transcription factor expression during development. Transcription factors are valuable to the insect developmental neurobiologist as cell specific markers, especially when used in conjunction with other markers, and to those interested in neural evolution, as indicators of possible homology. Results presented will be drawn mainly from grasshoppers and *Drosophila* for the transcription factors hunchback, even-skipped, engrailed, Antennapedia, and a probable homolog of deadpan.

06-009

EMBRYONIC DEVELOPMENT OF THE *DROSOPHILA* BRAIN

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We have characterized embryonic brain development in *Drosophila melanogaster* at the cellular and molecular level using antibody probes, enhancer detector strains, and mutant analysis. During embryogenesis, two bilaterally symmetrical cephalic neurogenic regions form. The brain commissure which interconnects these two brain hemispheres is pioneered by axons which project towards the midline in close association with an interhemispheric cellular bridge. The descending longitudinal pathways which connect the brain to the ventral nerve cord are also prefigured by a cellular bridge; pioneering descending and ascending neurons grow along these structures.

We have also studied the roles of the homeobox genes *orthodenticle (otd)* and *empty spiracles (ems)* in embryonic brain development of *Drosophila*. The embryonic brain is composed of three segmental neuromeres. The *otd* gene is expressed predominantly in the anterior neuromere. Expression of *ems* is restricted to the two posterior neuromeres. Mutation of *otd* eliminates the first (protocerebral) brain neuromere. Mutation of *ems* eliminates the second (deutocerebral) and third (tritocerebral) neuromeres. *otd* is also necessary for development of the dorsal protocerebrum of the adult brain. Thus, these homeobox genes are required for the development of specific brain segments in *Drosophila*. The regionalized expression of their homologs in vertebrate brains suggests an evolutionarily conserved program for brain development.

06-011

CELL MIGRATION IN THE EMBRYONIC NERVOUS SYSTEM.
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During embryogenesis, many cells in the developing nervous system undergo substantial rearrangements by the process of active migration along pre-defined pathways. In insects, cell migration is particularly important during the formation of the enteric nervous system (ENS) that innervates the gut. In the tobacco hornworm, *Manduca sexta*, we have shown that an identified population of undifferentiated neurons (named the EP cells) must migrate along specific muscle band pathways on the gut surface during normal development. Manipulations that inhibit migration cause severe disruptions of the ENS and prevent the gut from being innervated properly. To understand the mechanisms that control this process, we are investigating the nature of specific pathway molecules that guide neuronal migration, and the signal transduction systems by which the EP cells recognize and respond to these cues. One pathway molecule that appears to be required for migration is fasciclin II (MFas II), a cell adhesion receptor that is expressed by both the neurons and the muscle bands during migration. Experiments in embryo culture suggest that MFas II may promote migration via homophilic interactions between receptors on the neurons and muscle cells. We also have found that the onset of migration requires the activation of tyrosine kinases in the EP cells, which may be directly linked to MFas II receptors in the ENS. Other molecules (as yet unidentified) that are associated with non-pathway regions on the gut surface appear to inhibit migration. A second intracellular signaling protein, the heterotrimeric G protein Go  , is also expressed in the EP cells. However, stimulation of Go   causes an inhibition of migration in a calcium-dependent manner, suggesting that Go   may be part of the inhibitory response to non-pathway cues on the gut surface. The activity of these signaling molecules must ultimately regulate cytoskeletal assembly to affect migratory behavior. Using inhibitors of the cytoskeleton, we have shown that both migration and axon outgrowth by the EP cells require actin assembly, but migration is independent of tubulin assembly. We are now exploring how multiple signaling events may be integrated within a developing neuron to regulate its behavior and differentiation. Supported by NIH NS35369.

06-012

ASSOCIATION OF THE NITRIC OXIDE/CYCLIC GMP SYSTEM WITH SYNAPSE FORMATION IN INSECT NEURONS

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Immunocytochemical studies on embryos of *Locusta migratoria* show that many developing neurons go through a phase during which exposure to nitric oxide (NO) can stimulate production of cyclic GMP. This NO responsiveness is typically transient. It always begins after the neuron's axonal growth cone has arrived at its target and usually wanes after synaptic sites have been established. A wide variety of motoneurons, interneurons and sensory neurons show this property. Similar phenomena are evident in other insects such as the silverfish, *Ctenolepisma longicaudata*, the moth, *Manduca sexta*, and *Drosophila melanogaster*. In holometabolous insects like *Manduca* and *Drosophila* neurons show a second period of NO responsiveness at metamorphosis. This is seen both in larval neurons that are establishing new metamorphic contacts and in imaginal neurons that are making connections for the first time. Experiments in *Drosophila* using inhibitors of NO synthase result in neurons that fail to make their proper synaptic connections. We suggest that NO and cGMP constitute part of a retrograde signalling pathway that conveys signals to a neuron from its target thereby allowing the neuron to terminate axon elongation and initiate the processes of maturation and synaptogenesis.

06-014

SPECIES- AND STRAIN-SPECIFIC ANTENNAL LOBE EVENTS MEDIATING SEX ATTRACTION BY PHEROMONES IN MOTHS

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Mating in moths normally relies on long distance attraction of males by female-produced sex pheromones. The individual pheromone components are detected by different physiological types of receptor neurons on the male antenna. In many moths, however, the sex pheromone mediated behaviour has been shown to depend heavily on the precise ratio between the components of the pheromone blend. No interactions between pheromone components have been observed at the level of the peripheral receptors. Such interactions have been shown by intracellular recordings from antennal lobe interneurons of several moth species.

We have studied three species of moths with respect to blend recognition by antennal lobe interneurons. In two of the species, *Agrotis segetum* and *Ostrinia nubilalis*, the existence of populations with distinct differences in pheromone blends offers an excellent opportunity for comparative studies of the physiological basis of differences in behavioural response to pheromones. In *Trichoplusia ni*, we investigated the physiological correlates of a well established case of redundancy in the multi-component pheromone blend. In the turnip moth, *A. segetum*, antennal lobe interneurons responding specifically to the different blends of the two geographically distinct populations studied were found. The blend of females of the same population as the male under investigation often elicited a stronger neural response. In the European corn borer, *O. nubilalis*, the two pheromone strains that produce reversed ratios of the pheromone components (Z)- and (E)-11-tetradecenyl acetate, were shown to possess proportionally more antennal lobe neurons activated by the major component of the pheromone. Thus recordings from males of the so called Z-strain revealed more Z responding neurons, while recordings from males of the E-strain showed more E responding neurons. When antennal lobe neurons of the male cabbage looper moth, *T. ni*, were contacted intracellularly, blend-specific neurons were encountered. The significance of these neurons for the redundancy phenomenon in the pheromone communication was, however, ambiguous. In conclusion, we provide further support that male moths possess antennal lobe interneurons capable of recognizing pheromone blends and even discriminate between blends containing different ratios of the individual pheromone components detected by the peripheral receptors

06-013

LOW-LEVEL VISION IN HONEYBEES: SIMPLE STRATEGIES FOR FLIGHT CONTROL AND NAVIGATION

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Recent research has uncovered a number of different ways in which bees use low-level vision for navigational purposes. The distance flown to a food source is gauged by integrating the apparent motion of the visual world that is experienced en route. In other words, bees possess a visually driven "odometer" that is robust to variations in wind load and energy expenditure. Bees flying through a tunnel maintain equidistance to the flanking walls by balancing the apparent speeds of the images of the walls. This strategy enables them to negotiate narrow passages or to fly between obstacles. The speed of flight in a tunnel is controlled by holding constant the average image velocity as seen by the two eyes. This avoids potential collisions by ensuring that the bee slows down when flying through narrow passages. Bees landing on a horizontal surface hold constant the image velocity of the surface as they approach it. This automatically ensures that flight speed decreases with altitude, and is close to zero at touchdown. The movement-sensitive mechanisms underlying these various behaviours seem to be different, qualitatively as well as quantitatively, from those mediating the well-investigated optomotor response.

06-015

CENTRAL NEUROSECRETION AND NEUROMODULATION IN THE MOTH *MANDUCA SEXTA* (LEPIDOPTERA: SPHINGIDAE)

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As in other insects, the central nervous system (CNS) of *Manduca sexta* exhibits a variety of identifiable neurons that contain, and presumably secrete, neuropeptides and monoamines. Putative neuropeptides may be localized in neurons that project to neurohaemal release sites, in paracrine neurons that apparently subserve focal neurosecretion in the CNS, or in interneurons, colocalized with conventional neurotransmitters and possibly serving as modulatory cotransmitters. Monoamines such as dopamine, octopamine, and 5-hydroxytryptamine (5HT) are found in neuroendocrine cells, peripherally-projecting modulatory neurons, and local and widefield interneurons. It is likely that many of these types of neurons exert local or global neurosecretory influences through various mechanisms of neuromodulation and hence are important for the orchestration of behavior. As a case-in-point, this presentation will focus on a pair of identified, widefield, 5HT-containing interneurons in the deutocerebrum. Morphological investigations of these neurons suggest that they provide centrifugal feedback to the antennal lobes. Neurophysiological studies of the effects of 5HT on antennal-lobe neurons *in situ* and *in vitro* indicate that this monoamine exerts modulatory, and mainly or exclusively "up-regulatory," effects, such as broadening of action potentials and increased excitability of AL interneurons. The possibility that these 5HT-containing neurons might mediate state-dependent changes in olfactory function, and hence in odor-dependent behavior, will be discussed.

This line of research in our laboratory has been supported by NIH grant AI-23253 (to JGH), a Whitehall Foundation grant (to NTD), DFG grant KI 762/1-1 (to PK), a USA/NZ Cooperative Science Program grant (to ARM), and a fellowship from the UA Center for Insect Science (to X-JS).

06-016

A UNIQUE MEMBER OF THE PHEROMONE-BINDING PROTEIN SUPERFAMILY COMMONLY FOUND IN THE TASTE AND OLFACTORY SYSTEMS OF THE FLY AND ITS POSSIBLE FUNCTION.

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An acidic 14 kDa protein purified in the blow-fly, *Phormia regina* belonged to the insect pheromone-binding protein superfamily, but unlikely other members of the superfamily, it was distributed in both taste and olfactory organs. A similar protein was also isolated in *Drosophila melanogaster*. Considering their distribution, cDNA sequences and structural features, we concluded that the proteins belongs to a unique subfamily whose members have convergently evolved for a common function required for both senses of taste and olfaction. By an electrophysiological experiment using antiserum, we also suggested that these proteins carry fragrant components of natural foods in taste system as well as in olfactory systems.

06-018

PRINCIPLES OF ACOUSTIC COMMUNICATION IN TETTIGONIDS

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Tettigoniids perform a pronounced species-specific acousto-vibratory communication which is used in female attraction, in intraspecific male competition but also in spatial distribution of the individuals of a population.

The complex tibial organs of all six legs and the combined auditory-vibratory sensory system of the CNS establish the receiver of this communicatory system, which detects and processes the sound signals emitted by conspecific males.

Recent studies on the receptor organs of different species have revealed that during the evolution of the various species of tettigoniids the sound conducting system of the auditory organs was more readily adapted than the receptors themselves to detect the frequencies in the conspecific song.

06-017

TRANSMITTER PLASTICITY IN INDIVIDUAL PEPTIDERGIC NEURONS AND ITS REGULATION BY STEROID HORMONES.

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The tobacco hawkmoth *Manduca sexta* contains 4 lateral neurosecretory neurons (LNCs) that undergo a change in morphology and biochemistry during metamorphosis. Several different assays, including single cell bioassays, have determined that the LNCs in larvae express high levels of Cardioacceleratory Peptides (CAPs) and low levels of bursicon. In contrast bursicon expression increases significantly in adult LNCs while CAP levels are below detection. Several lines of evidence suggest that this transmitter switch is under the regulation of the insect ecdysteroid 20-hydroxyecdysone (20-HE). Results from *in vivo* endocrine manipulations indicate that the two 20-HE pulses during the last larval instar affect different aspects of this switch, with the commitment pulse (CP) causing a decline in CAP levels and the subsequent prepupal peak (PP) triggering an increase in bursicon levels. To evaluate the morphological changes seen *in vivo*, individual LNCs from feeding Vth instar larvae were placed in an *in vitro* culture system and exposed to different hormonal regimens for up to two weeks. The morphology of the cultured LNCs was concentration dependent; primary neurite outgrowth was stimulated at CP levels of 20-HE while secondary and tertiary branching was promoted in a concentration of 20-HE that mimics the PP. In this talk I will discuss the experiments leading to these results and the behavioral implications of this plasticity.

06-019

FUNCTIONAL CHARACTERIZATION OF CLONED *DROSOPHILA MELANOGASTER* NEUROTRANSMITTER RECEPTORS BY EXPRESSION IN STABLE *DROSOPHILA* CELL LINES.

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Application of molecular cloning and genetics to the investigation of chemical neurotransmission in *Drosophila melanogaster* has resulted in the identification of many putative neurotransmitter receptor encoding genes. Multiple subunits of *Drosophila* ionotropic receptors for GABA, acetylcholine (nicotinic) and L-glutamate, have been identified. G-protein coupled receptors for acetylcholine (muscarinic), 5-HT, octopamine/tyramine and dopamine, have been cloned, and subtypes of several of these *Drosophila* metabotropic receptors have also been identified. The demonstration of functional expression using such methods as electrophysiology, calcium imaging and enzyme assays is an essential step in ascribing roles for cloned, putative neurotransmitter receptors. Expression systems used for *Drosophila* receptors include transient and stable expression in mammalian cells lines, transient expression in *Xenopus* oocytes, transient and constitutive expression in the baculovirus/Sf9 cell expression system. To overcome some of the limitations of expression vehicles used to date, we have generated *Drosophila* S2/M3 cell lines, stably expressing cloned *Drosophila* neurotransmitter receptors, using the plasmid pRmHa3 (containing the metallothionein promoter of *Drosophila*), and a selection plasmid pCoHygro (containing a bacterial hygromycin B phosphotransferase gene). In this way, cell lines have been generated expressing the wild-type and dieldrin-resistant forms of a *Drosophila* ionotropic GABA receptor subunit (RDL) that forms robust homo-oligomers¹ and muscarinic acetylcholine receptors². Thus, the procedure is effective for ionotropic and G-protein coupled *Drosophila* receptors, and lines expressing several other cloned *Drosophila* neurotransmitter receptors are now being characterized. Cell lines facilitate detailed characterization of *Drosophila* receptor subtypes which is often difficult in native membranes containing mixed receptor populations. They permit studies of the interactions of selected neurotransmitter receptors and the intracellular signalling pathways they activate. They also offer improved understanding of both established and novel molecular targets for insect control agents, and may be useful in the generation of high-throughput screening assays.

1) Millar, N.S., Buckingham, S.D. and Sattelle, D.B. (1994) Proc.R.Soc.(Lond.)B 258, 307-314.

2) Millar, N.S., Baylis, H.A., Reaper, C., Bunting, R., Mason, W.T. and Sattelle, D.B. (1995) J.exp.Biol. 198, 1843-1850.

06-020

MOLECULAR BIOLOGY AND EXPRESSION OF NOVEL INSECT GABA RECEPTOR SUBUNITS

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Using the unique approach of isolating drug (PTX) and pesticide (cyclodiene insecticide) resistant *Drosophila* mutants we have cloned a novel GABA receptor subunit gene *Resistance to dieldrin* or *Rdl*. *Rdl* is very different from vertebrate GABA_A receptors because it 1) forms homomultimeric GABA gated chloride ion channels in heterologous systems with high efficiency, 2) shows more extensive alternative splicing, 3) has a different genomic organization and 4) possesses higher sequence identity to glycine receptors than GABA_A receptor subunits themselves (although it does not respond to glycine as an agonist). We have previously shown that resistance is associated with a single alanine> serine replacement within M2 the region proposed to line the chloride ion channel pore (*Nature*, 363, 449, *PNAS*, 90, 1957) and that the mechanism of resistance is brought about by a unique combination of direct changes in the binding site and allosteric destabilization of the drug preferred desensitized state (*J. Physiol.* 479.1, 65). Here we present *in situ* hybridization data on the spatial and temporal expression of *Rdl* (*Invertebrate Neuroscience*, 1, in press) with that of a second *Drosophila* GABA receptor subunit that appears to be a homolog of vertebrate GABA_A receptor β subunits.

06-022

NEUROPEPTIDE BIOSYNTHESIS: MOLECULAR MECHANISMS AND NEW INSECTICIDE TARGETS

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We are using the corpora cardiaca (CC) of the locust *Schistocerca gregaria* as a model system to study the molecular mechanisms of insect neuropeptide biosynthesis. The glandular CC are particularly homogeneous, containing approximately 6-10,000 intrinsic neurosecretory cells which are devoted to the biosynthesis of the two adipokinetic hormones (AKH I and II) and a set of three dimeric neuropeptides called AKH Precursor Related Peptides 1, 2 and 3 (or APRP 1, 2, 3). These five products are generated by the post-translational processing of two related monomeric prohormones. We have determined the temporal order and the intracellular location of these processing events, as well as the biochemical mechanisms by which the processing reactions are accomplished. In addition, we have reconstituted *in vitro* the steps in proteolytic processing of the dimeric AKH I precursor.

Using NMR spectroscopy we have determined the solution structure of the AKH I precursor in hopes of identifying the structural features of the precursors which govern processing endopeptidase specificity. By modifying the structure of the precursor in the vicinity of the processing site we are able to either prevent processing or not. Such experiments suggest that an Ω -loop structure is important in molecular recognition by the endopeptidase. Our work may provide the basis for the design of specific enzyme inhibitors to be used as lead compounds for new insecticides.

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06-021

SHAKING-B IS REQUIRED FOR THE ESTABLISHMENT OF ELECTRICAL SYNAPSES IN THE *DROSOPHILA* GIANT FIBRE SYSTEM.

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The establishment of the highly specific synaptic connections between neurons must require the expression of particular genes. One candidate is the *Drosophila shaking-B* locus. *shaking-B* was discovered by screening for mutations that abolish the fly's escape response, a behaviour mediated by a network of identifiable neurons known as the giant fibre system (GFS) (Thomas & Wyman, 1984. *J. Neurosci* 4: 530-538).

Genetic analysis has revealed that at least 2 distinct functions reside at the *shak-B* locus, one being required for viability and the other for the establishment of some GFS electrical synapses. We have cloned *shaking-B* and have isolated cDNAs reflecting multiply spliced transcripts. The characterisation of different mutations has enabled us to distinguish between transcripts required for essential and neural *shak-B* functions (Crompton et al., 1995. *Dev. Biol.* 170: 142-158).

We are examining the role of *shak-B* in synapse formation. Using an enhancer trap line, we followed the development of the giant fibre, the major descending neuron of the GFS pathway and injected Lucifer Yellow intracellularly to describe and to map the development of the electrical synapses it forms. Immunocytochemistry with *Shak-B* peptide antisera demonstrates that the protein is first localised to the region of the GF synapses at about the time of synaptogenesis (Phelan et al., *J. Neurosci.* in press). We see no corresponding expression in neural *shak-B* mutants in which we show that all of the electrical synapses in the GFS are dye uncoupled.

shak-B function is clearly required for the establishment of electrical connections. The protein may itself be a structural component of synaptic gap junctions or play a role in the assembly and/or stabilisation of the junctional complex.

06-023

NEUROPEPTIDE BIOSYNTHESIS: MOLECULAR GENETIC ANALYSIS IN *DROSOPHILA*.

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We are studying neuropeptide biosynthesis in *Drosophila* to employ genetics in defining mechanisms that regulate the appearance and use of neuropeptides. We have defined two questions relating to this problem.

The first considers the transcriptional mechanisms that restrict neuropeptide gene expression to a small subset of CNS neurons. The *FMRamide* neuropeptide gene is expressed in ~50 diverse neurons. Using transgenic animals, we have demonstrated both positive and negative transcriptional regulation of this gene. Further, our results indicate the existence of cell-specific enhancer elements within the *FMRamide* promoter, that are small and that may bind specific sets of transcriptional regulators. To date, we have found two such enhancers (size: 170 and 190 bp, respectively); they regulate reporter expression in 2 of the ~15 different cell types that normally express the *FMRamide* gene.

The second question considers the processing of neuropeptide precursors by enzymes. 50% of all neuropeptides are amidated at their C termini: this event is the final step in neuropeptide biosynthesis. Although *Drosophila* contains both enzymatic activities necessary to achieve amidation, to date we have only found gene sequences for the first step, hydroxylation of C terminal glycine residues. This single copy gene is expressed in a wide variety of tissues; in the CNS, it is highly expressed in identified neuropeptide neurons, and weakly expressed throughout the tissue. We are currently searching for mutations in this gene with which to evaluate its *in vivo* contribution to neuropeptide biosynthesis.

06-024

FUNCTION OF INSECT EPITRACHEAL ENDOCRINE SYSTEM

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A novel, segmentally distributed endocrine system of epitracheal glands (EG) in moths and other insects is implicated in the control of ecdysis. In moths, individual glands are attached to the trachei near each spiracle and are composed of a large peptidergic Inka cell and 2-3 smaller glandular cells. Before initiation of ecdysis, Inka cells are white and show strong immunoreactivity with antibodies to small cardioactive peptide B and FMRFamide. Upon initiation of ecdysis, Inka cells decrease in size and lose their white colour and immunoreactivity. Injection of EG extracts into pharate larvae, pupae and adults up to 1-7 days prior to natural ecdysis triggers pre-ecdysis followed by ecdysis behaviour within 2-10 min. Exposure of the isolated central nervous system (CNS) to EG extract triggers motor bursting patterns similar in frequency and duration to pre-ecdysis and ecdysis behaviours observed *in vivo*. We isolated novel "ecdysis triggering hormones" (ETH) from *Bombyx mori* and *Manduca sexta* EG with potent ecdysis activity *in vivo* and *in vitro*. ETH is produced solely by Inka cells as indicated by immunohistochemical staining with antiserum specific against ETH N-terminal. Inka cells also produce a set of nonrelated peptides with ETH-like activity and FMRFamide-like peptides (FLP), which are expressed by different genes during intermolt period and released at the end of each instar. Expression of these genes and development of the CNS sensitivity to ETH seems to be regulated by ecdysteroids, while release of the ETH and FLP from Inka cells is likely regulated by eclosion hormone. ETH may directly act on the CNS as an immediate blood borne trigger for ecdysis, while other Inka peptides regulate events associated with this behaviour.

06-026

THE *DROSOPHILA* BRAIN: FROM TWO- TO THREE-DIMENSIONS

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Drosophila melanogaster is the organism of choice for genetic analyses of function and, especially, development of the nervous system. The ready availability of molecular probes in *Drosophila* provides powerful new methods to examine and visualise the brain's anatomy. These are often from the patterns of nuclear localisation of gene products, however, and must be integrated into the anatomy of pathways and identified neurons, which are mostly described from the pattern of neurites rather than the positions of the somata. Most recent studies address the adult brain, but the need for similar approaches in the little-studied larval brain is no less acute. The ease with which the larval brain can be dissected out, and its handy dimensions at this stage of the life-cycle, have promoted the recent widespread use of the larval CNS in wholemount preparations. These are being widely used to screen and view patterns of gene expression in enhancer trap lines, especially GAL4 lines. We have reconstructed the 3-D surfaces of the brain and neuropil regions, from profiles in consecutive semithin sections stained by the osmium-ethyl gallate method, using a Silicon Graphics computer and ICAR 5.0.1 (ISG Technologies Inc., Canada). Images are grabbed from micrographs aligned, *seriatim*, using AMICUS (ISG) to compare each image with others, obtaining the best fit by eye. Virtual sections of the reconstructed brain can then be compared with views of the larval brain derived from confocal image stacks of intact, wholemount brains stained with propidium iodide and other cellular markers. Such comparisons not only validate the accuracy of the reconstructions, but also allow neuropil regions and their fibre tracts to be recognised in orthogonal section planes from their profiles identified in the section plane reconstructed in 3-D. These approaches are preliminary to localising the staining patterns, or expression domains of genes, to the somata of specific cells or cell populations, from material sectioned in a variety of planes by various means (from frozen sections to confocal images). Even though some regions of the larval CNS, especially the developing optic lobes of the imaginal brain, pose a significant challenge to the ability of computer imaging methods to derive reconstructions at single-cell resolution, the latter are nevertheless also a first step to a developmental series of such reconstructions. Such series are essential for time-lapse views both of gene expression and of regional morphogenesis. Supported by grants from NSERC: CPG 0181755 and EQM0153222.

06-025

THE INSECT BRAIN: AN EVOLUTIONARY ACCOMPLISHMENT IN MINIATURIZATION AND CONVERGENCE.

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That the biomass of insects outweighs all other organisms may be less of a testimony to their evolutionary success than the remarkable sophistication of their central nervous systems. Amongst arthropods, insect brains shows the closest resemblance to those of vertebrates, particularly with respect to the organization of their visual, olfactory, and leg motor neuropils. One phylogenetically ancient higher center, the mushroom bodies, demonstrate an evolutionary jump in the pterygote insects, becoming a double pair rather than singly paired as in apterygotes and other arthropods. In certain of the larger Orthoptera and Hymenoptera, these paired double centers can comprise nearly a million nerve cells. Functions ascribed to them are reminiscent of functions ascribed to mammalian pyriform cortex, hippocampus, and neocortex. Centers that are characteristic of insects, such as the central body complex, suggest comparisons with the cerebellum. At the output level, the organization of descending pathways onto motor centers suggests population coding, a read-out mechanism thought to be typical of vertebrate CNS. In this talk I shall outline a few such examples from insect brains which, as exclaimed by Ramon y Cajal 80 years ago, make those of vertebrates look crude by comparison.

06-027

THE ANTENNAL LOBE OF *DROSOPHILA MELANOGASTER*

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The antennal lobe (AL) of *D. melanogaster*, the primary olfactory association center, consists of 30-40 glomeruli. It derives from a non-glomerular larval precursor. Five of the glomeruli in the adult AL are exclusive targets of the ipsilateral antenna, the remaining are bilateral. Individual afferent fibers are glomerulus-specific. Sensory projections appear to be ruled by sensillum type rather than topographically.

Local interneurons (LI) of the AL have lateral cell bodies and establish arborizations in all of the glomeruli. Relay interneurons (RI) connect individual glomeruli with the calyx of the mushroom bodies (MBs) and with the lateral protocerebrum (LPR). Their cell bodies are located either anterodorsal, lateral or ventral to the AL. AL interneurons derive from a single pair of lateral neuroblasts that begins to divide early in larval life, and from others that initiate mitosis later, with a peak of 10-12 pairs of dividing neuroblasts in the late 3rd larval instar.

Application of hydroxyurea (HU) to early 1st instar larvae leads to the loss of the MBs and to a volume reduction of the AL¹. The latter changes are due to HU-induced ablation of the lateral neuroblast¹. To identify the elements affected by this procedure, we applied HU to GAL4 enhancer trap lines expressed in specific types of AL interneurons.

Untreated flies of line *GH146* express GAL4 in RI and in 30-50 fibers extending through the MBs. HU application leads to the loss of RI with lateral cell bodies only. Although obvious MBs are lacking, 30-50 fibers occupy positions reminiscent of the MBs. From the surviving RI, axons extend to the LPR, but do not form side-branches in the presumed calyx region. Line *GH298* expresses GAL4 in LI, elements which become completely ablated by HU.

These data suggest that LI and the RI with lateral cell bodies derive from the early-dividing lateral neuroblast, whereas the remaining interneurons are formed by neuroblasts that undergo mitosis later on in larval life. HU severely affects the MBs, without ablating them completely.

¹ DeBelle S, Heisenberg M, Science 263, 692, 1994

06-028

STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE *DROSOPHILA* MUSHROOM BODIES

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The two mushroom bodies (MBs) in the *Drosophila* brain consist each of about 2500 Kenyon cells that are derived from 4 neuroblasts proliferating during embryonic and larval stages. MBs show a high degree of structural plasticity. Kenyon cells shed and regrow their fibers during metamorphosis. Fiber number in the peduncle and the volume of the calyx depend on the growth conditions during larval stages and on sensory experiences during adulthood. Our aim is to study the molecular and cellular mechanisms underlying the process of structural plasticity. We use Gal-4 enhancer trap lines that label specific subsets of Kenyon cells allowing us to follow them during development. Several mutations have been isolated that interfere with normal mushroom body development. For three mutations we have started the molecular characterization of the corresponding genes.

MBs are required for normal odor discrimination learning. In the mutant *mushroom body miniature*¹ (*mbm*¹) acquisition but not memory is affected (deBelle and M.H., in prep.) For colour and pattern discrimination learning MBs are dispensable, irrespective of whether the training is classical or operant. However, in flies without MBs, pattern discrimination learning can be abolished by 220ms dark periods during training (R.Wolf and M.H., Neurobiology Conference, Göttingen 1996). This finding suggests that the role of MBs in learning needs to be reconsidered.

06-030

THE SUBOESOPHAGEAL GANGLION AS A NEUROSECRETORY CENTER IN INSECTS

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The general believe about the suboesophageal ganglion (SOG) of insects is that its only function is the coordination of the mouthparts. More recent work has shown, that apart from this task the SOG also represents a higher order motor center for the coordination of walking, flight, respiration, stridulation, and the generation of neck movements. Even more recent is the notion that the SOG is involved in neuroendocrine control to an extent that was previously unsuspected. Next to the brain, the SOG appears to contain the largest number of neurosecretory cells. Morphologically complex types of neurosecretory cells project into the entire central nervous system, the retrocerebral glandular complex, the lateral heart nerves, or form neurohaemal networks on the surface of peripheral nerves. Additional data even give hints that the SOG is fourth functional complex: neuronal and/or neuroendocrine control of the foregut.

06-029

THE CENTRAL COMPLEX: EVIDENCE FOR A ROLE IN POLARIZED-LIGHT ORIENTATION

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The central complex (CC) comprises a group of neuropils in the center of the insect brain. It consists of the protocerebral bridge, the upper and lower division of the central body and the paired noduli, and is closely connected to the lateral accessory lobes (LAL) of the brain (reviewed by Homberg 1987, in AP Gupta [ed] *Arthropod brain*, Wiley, New York, pp 347-367). We have examined the organization and functional role of the central complex in the locust *Schistocerca gregaria*.

The CC communicates with the ventral nerve cord via ascending and descending neurons to and from the LAL. These neurons as well as many CC neurons show activity changes associated with stationary flight, suggesting a role of the CC in certain aspects of flight control (Homberg 1994, *J Comp Physiol A* 175:597-610). Two pathways, the posterior and anterior optic tracts, provide visual input to the CC and LAL. Many and perhaps all neurons associated with the lower division of the central body are sensitive to polarized light delivered to dorsal areas of the compound eyes. The responses of these neurons show polarization opponency, i.e. e-vectors eliciting maximal tonic excitation are oriented 90° to e-vectors causing maximal tonic inhibition. Some inputs to the CC originate in the accessory medulla of the optic lobe, a neuropil which has been implicated with the circadian pacemaking system (Würden and Homberg 1995, *J Comp Neurol* 362:305-319). These inputs might serve for time compensation in polarized-light processing in the CC. Taken together, the data suggest that a subsystem of the CC serves a role in compass orientation of the locust with respect to the sky polarization pattern.

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06-031

EXPRESSION AND FUNCTION OF DROMYOSUPPRESSIN IN THE *DROSOPHILA* BRAIN.R. Nichols, M. Dickerson, J. McCormick, and K. Paisley
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We have isolated and characterized the dromyosuppressin peptide (TDVDHVFLRFamide, DMS). DMS is an abundant peptide expressed in the brain and gastrointestinal tract at all stages of development. DMS immunoreactive processes project from neurons in the superior protocerebrum to innervate the anterior portion of the dorsal vessel.

We have established an *in vivo* assay and demonstrated that DMS inhibits *Drosophila* heart rate in a dose dependent manner. The amino acid residues critical for cardioinhibitory action have been identified using the method of alanine scan. We are utilizing a pharmacological and genetic approach to determine the mechanism of action of DMS.

The dromyosuppressin gene (*Dms*) has been isolated and cytologically localized to the right arm of the third chromosome at 96A1.

06-032

**NEUROPEPTIDES IN THE INSECT BRAIN: WHAT ARE
THEIR MESSAGES?**

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A multitude of neuropeptides are known to be synthesized by neurons and neurosecretory cells of the insect brain. At present we have no information about the physiological functions of any one of these peptides within the brain itself, but for some of them hormonal actions have been determined or at least indicated. Two families of myotropic insect neuropeptides have been selected as examples to illustrate the diversity of putative neuropeptide actions: the leucokinins (LKs) and the tachykinins (TKs). In most insect species analyzed to date the LKs are likely to be utilized both as neurohormones and neuromodulators/co-transmitters, whereas the TKs appear to be only neuromodulators/co-transmitters. In the brain of the cockroach *Leucophaea maderae*, both the LKs, and the recently isolated TKs are found in several isoforms. Immunocytochemistry (ICC) has given indications of putative sites of release of the LKs and TKs in *L. maderae*. The LKs (probably all eight isoforms) can be released from corpora cardiaca and may act as diuretic and myoactive hormones. In the brain LKs are present in distinct sets of neurons and may have defined actions as regulators of behaviour. Also in interneurons several, if not all, LK isoforms appear co-localized. The TKs on the other hand are distributed in larger numbers of diverse neuron types and appear more likely to be utilized as co-transmitters with distributed functions. Co-localization of different the TK isoforms has not yet been determined for *L. maderae*, but has been shown for some neurons in *Locusta migratoria*. A brief comparison between *L. maderae*, *L. migratoria* and *Drosophila melanogaster* is made to highlight some preserved and some variable features of the neuronal and neuroendocrine systems utilizing leucokinin- and tachykinin-like peptides.

06-033

PHOTOPERIODIC RECEPTOR IN *RIPTORTUS CLAVATUS*: REGIONAL DIFFERENCE IN THE COMPOUND EYE

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The bean bug, *Riptortus clavatus* (Thunberg) (Heteroptera: Alydidae) shows a long-day photoperiodic response that controls adult diapause. The location of photoreceptors for the photoperiodism was examined in *R. clavatus* by complete or partial removal of photoreceptor organs. Even after one compound eye or both ocelli were removed, the insects were sensitive to photoperiod. After both compound eyes were removed, however, the insects reproduced regardless of the photoperiod. Therefore the photoreceptors for photoperiodism were not in the ocelli but in the compound eyes. To clarify whether the ommatidia in the compound eye have a regional difference in the reception of photoperiod, photoperiodic sensitivity was examined after one compound eye and a part of the contralateral one were removed. Only when the central region of the compound eye was removed, the insects lost the photoperiodic sensitivity. Therefore the ommatidia in the central region of the compound eye play the principal role in the reception of photoperiod.

06-035

ODOUR-GUIDED HOST-FINDING BY HAEMATOPHAGOUS MOSQUITOES: IDENTIFICATION AND NEURAL ENCODING OF HOST ODOURS (DIPTERA; CULICIDAE).

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In the process of odour-guided host-finding mosquitoes perceive volatiles by different kinds of sensory receptors, situated on the antennae and palps. Sensitivity and specificity of the olfactory receptors of the mosquito *Anopheles Gambiae* s.s., an important vector of human malaria, were studied by using electrophysiological methods.

Responses of single olfactory neurons were recorded to mixtures of fatty acids known to be attractive in windtunnel bio-assays as well as to individual fatty acids. Sensitive neurons for short chain fatty acids were found in characteristically shaped medium sized sensilla trichodea, a type abundant on the female antenna.

Furthermore, sensory responses were used to assist the identification of potential host attractants by combining a gas chromatograph with electrophysiological set-ups for electroantennogram (EAG) or single cell (SC) recordings.

Scanning electron microscopy studies on the antennae of *An. gambi-ae* were performed to supplement electrophysiological studies with morphological information. Preliminary observations indicate that the sensilla trichodea (known to house olfactory receptors) differ from those of other well-studied *Anopheles* species, like *An. stephensi*.

06-034

PHOTOPERIODIC RECEPTOR IN THE BAND-LEGGED GROUND CRICKET, *PTERONEMOBIUS NIGROFASCIATUS*

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In *Pteronemobius nigrofasciatus* Walker (Orthoptera: Gryllidae), the induction of egg diapause is controlled by a long-day photoperiodic response in the parental generation and the critical daylength in our strain (Osaka, Japan) is between 13 and 14 hr at 25°C. When crickets were reared under a short-day (LD 12:12) photoperiod and then transferred to a long-day (LD 16:8) photoperiod upon adult emergence, the adults mainly laid nondiapause eggs. However, adults maintained continuously under short-day conditions laid diapause eggs. When compound eyes were bilaterally removed after adult emergence, the crickets mainly laid nondiapause eggs, irrespective of the photoperiod. Thus, the adults completely lost their sensitivity to photoperiod after bilateral removal of their compound eyes. Unilateral removal of the compound eye also affected the crickets under a short-day photoperiod, and the incidence of diapause eggs was intermediate between that laid by intact adults and that laid by adults after the bilateral removal of compound eyes. The incidence of diapause eggs in sham-operated crickets was not significantly different from that in intact crickets under both sets of photoperiodic conditions. These results show that *P. nigrofasciatus* use the compound eyes as a photoperiodic receptor.

06-036

OLFACTORY SENSILLA AND RECEPTOR NEURON RESPONSES OF *PHORACANTHA SEMIPUNCTATA* (COL.: CERAMBYCIDAE) TO HOST AND NON-HOST TREE COMPOUNDSO. Lopes¹, E. Barata¹, H. Mustaparta², J. Araujo¹¹ Departamento de Biologia, Universidade de Évora, Évora, Portugal² Department of Zoology, University of Trondheim, Norway

Phoracantha semipunctata is a wood borer longhorned beetle specific of *Eucalyptus*. The odour of *E. globulus* foliage and logs is an important orientation cue in the host finding behaviour of this insect. Olfactory sensilla on the antennal flagellum of *P. semipunctata* could be distinguished with scanning and transmission electron microscope. They were smooth, thin and multiporous - walled pegs. At least two olfactory receptor cells were identified with them, from which branched outer segments reach into the cuticular peg. "Head-space" volatiles evaporated from the foliage of *E. globulus* and from the foliage of the non-hosts *Pinus pinaster* and *Olea europaea* were separated in the gas chromatograph (GC) capillary column. The effluent was split at the end of the column, leading one half of the eluent to the GC-detector and the other half out of the GC-oven to the insect antenna during recording of receptor neurons activity associated with olfactory sensilla. In a sample of 42 receptor neurons, we defined 26 neuron types based on the number of compounds that elicited a response. We did not find neurons that responded to more than 4 different compounds. One neuron type responded specifically to a single not identified compound only present in the non-host *O. europaea*. The results suggest that the insect might have a population of neurons tuned to the reception of compounds only present in non-host species. These specific receptor neurons might enable the insect to a unambiguous detection of non-hosts and ready exhibition of avoidance behavior.

06-037

PLANT-ODOUR RESPONSE PROFILES OF APHIDS: ELECTROANTENNOGRAMS OF *MEGOURA VICIAE*, *APHIS FABAE*, *MYZUS PERSICAE* AND *BREVICORYNE BRASSICAE*

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In order to identify chemicals, which are involved in host location of aphids, a wide collection of plant odour components was screened on the antennal responses in four aphid species, i.e., the vetch aphid *Megoura viciae* Buckton, the black bean aphid *Aphis fabae* Scop., the peach-potato aphid *Myzus persicae* (Sulz.) and the cabbage aphid *Brevicoryne brassicae* (L.), as recorded by the electroantennogram technique. From the response profiles it is obvious that aphids, in general, show distinct sensitivities for (a) general green leaf volatiles, e.g., (*E*)-2-hexenal, (b) benzaldehydes, 4-methoxybenzaldehyde, (c) carvones, (-)-(*R*)-carvone (d) citronellal, (e) nitriles, hexano- and heptano-nitrile, and (f) isothiocyanates, butyl and 4-pentenyl isothiocyanate. Each of the aphid species differs in varying degrees from the general response profile. It is noteworthy that the response profiles cover potential attractants as well as suspected repellents. Furthermore, we studied (a) the response profiles of different aphid forms in *A. fabae* and *M. persicae*, (b) the response profiles of two clones in *M. persicae*, and (c) the effects of rearing conditions (host plants and artificial diet) for *M. persicae*.

06-039

ANTENNAL COCKROACH PROTEIN PHOSPHORYLATION IN THE COURSE OF THE TRANSDUCTION OF THE OLFACTORY MESSAGE

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Current evidence implicating second messengers such as inositol triphosphate or cGMP in molecular mechanisms underlying insect olfaction suggests that pheromonal signals act on specific protein phosphorylation. To further validate this hypothesis, we studied the effects of second messengers and pheromonal blend on phosphorylation of antennal proteins in the male cockroach *Periplaneta americana*.

-1- Proteins from adult male antennae were phosphorylated *in vitro* in the presence of [γ ³²P]-ATP, then separated by SDS-PAGE. A wide range phosphopolypeptides were visualized. Addition of Ca⁺⁺/calmodulin to the incubation medium enhanced phosphorylation of polypeptides with molecular weights of 38, 48, 51, 54 and 58 kDa. By stimulating PKC, Ca⁺⁺/Phosphatidyl-Serine (PS)/Phorbol Myristate Acetate (PMA) led to the appearance of three phosphopolypeptides of 36, 70 and 120 kDa. In the presence of cAMP, two polypeptides of 46 and 42 kDa appeared. The 42 kDa polypeptide also appeared in the presence of cGMP. Addition of spermine enhanced phosphorylation of various polypeptides including phosphopolypeptides of 23, 26/27, 28, 32, 38, and 54 kDa.
-2- Comparison of antennal polypeptide content with that in the cerci, leg, brain, and fat body showed that the 28, 36, and 48 kDa polypeptides were specific to antennae.
-3- *In vitro* phosphorylation of proteins obtained from antennae subjected to pheromonal stimulation for 16 seconds prior to homogenisation revealed inhibition of the 120, 70, 64, 38, 28, and 26/27 kDa polypeptides and strong stimulation of the 58, 54, 51, 48, and 32 kDa polypeptides. A 107 kDa polypeptide was observed only after pheromonal stimulation by Ca⁺⁺/PS/PMA.
These findings suggest that Ca⁺⁺ and PKC-dependent protein phosphorylation pathways play an important role in the transduction of pheromonal signals in antennae of male cockroach *P. americana*. They also suggest that polyamine-dependent phospho/dephosphorylations are implicated in the transmission of olfactory messages. We speculate that specific phosphoproteins modulate sensitivity and signal amplification during the olfactory transduction process.

06-038

SINGLE CELL RESPONSES IN MALE *Agrotis segetum* SEXUAL PHEROMONE PERIPHERAL RECEPTORS IN A WIND TUNNEL GENERATED PHEROMONE PLUME

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Single cell responses from male *Agrotis segetum* Schiff. (Lepidoptera: Noctuidae) pheromone receptors were recorded in a wind tunnel 1.5 meters downwind of a pheromone source. We used rubber septa and filter paper with single pheromone components / complete blends with loads varying between 0.001 - 1000 μ g. We also used excised female pheromone glands. Recordings were made during 2 minutes of exposure and the resulting signal was analysed for number of spikes, number of spike trains and mean number of spikes per spike train. We have established dose-response curves for Z5-10:OAc and Z7-12:OAc cells. In this way we were able to directly compare pheromone release rates from syntethic baits and female glands at close to natural levels. We also describe the plume structure from a single cell point of view.

06-040

SINGLE-SENSILLUM RESPONSES OF MALE *RHYACOPHILA NUBILA* (TRICHOPTERA; RHYACOPHILIDAE) TO POSSIBLE SEX PHEROMONE COMPONENTS

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This study for the first time shows responses of single receptor neurons on the antennae of male caddisflies (Trichoptera) to possible female sex pheromone components. As a sister order of the Lepidoptera, the Trichoptera have attracted some interest in the matter of pheromone communication. Pheromone systems similar to those found in primitive Lepidoptera have been demonstrated in Trichoptera, suggesting the possibility of a common origin. Studies of caddisflies and primitive moths could give an insight regarding the origin and evolution of the lepidopteran systems of pheromone communication. The aim of the present study is to gather information about the peripheral level of pheromone detection in Trichoptera, which could serve as a basis for phylogenetic comparisons with the corresponding apparatus in Lepidoptera, and as a stepping stone for further investigations of the neural integration of pheromone signals in Trichoptera.
Six compounds, heptan-2-one, (R)-heptan-2-ol, (S)-heptan-2-ol, nonan-2-one, (R)-nonan-2-ol, and (S)-nonan-2-ol were tested on the antennae of 14 male *Rhyacophila nubila*, with tungsten single-sensillum techniques. These compounds show EAD activity in the males and all except the two (S)-enantiomers are also produced by the females. Of 93 neurons examined, a majority showed a high sensitivity and were selectively tuned to one of the compounds (R)-heptan-2-ol, (S)-heptan-2-ol, or nonan-2-one. No neurons selectively tuned to the compounds heptan-2-one, (R)-nonan-2-one, or (S)-nonan-2-ol were found. The results strongly support the hypothesis that some of the tested compounds are part of the female sex pheromone blend. They also show that the males have the ability to differentiate between chiral isomers at the receptor neuron level. This suggests that the pheromone signal used in *R. nubila* could be very similar to the signal used in the lepidopteran family Eriocraniidae. This fact also provides further support for a relatedness between trichopteran and lepidopteran sex pheromone systems. The males possess receptor neurons sensitive to (S)-heptan-2-ol, a compound which is not produced by the conspecific females. The possible role of these receptor neurons could be to mediate an avoidance of heterospecific females.

06-041

CENTRAL NERVOUS PROCESSING OF AGGREGATION
PHEROMONES IN FIFTH INSTAR DESERT LOCUSTSR. Ignell, S. Anton, B.S. Hansson
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Adult and nymphal *Schistocerca gregaria* aggregate in response to two different releaser pheromone systems. Adult insects aggregate only in response to adult male produced odours, whereas the nymphal volatiles only elicit a behavioural response in the nymphs (Torto *et al.*, Chem Ecol 20:1749-1762, 1994; Obeng-Ofori *et al.*, Entomol Exp Appl 73:85-91, 1994). Like in the cockroach, *Periplaneta americana*, the desert locust show a drastic increase in the number of sensilla, containing specific pheromone receptor neurons, in the last moult (Prillinger, Cell Tissue Res, 215:563-575, 1981; Chapman and Greenwood, Int J Insect Morphol & Embryol 15:83-96, 1986). The afferent neurons synapse with an increasing number of antennal lobe interneurons. Antennal lobe neurons increase either through multiplication or differentiation (Chambille and Rospars, Int J Insect Morphol & Embryol 14:203-226, 1985, and references therein). The observed difference in response characteristics between adult and nymphs could thus be related to the differences in the olfactory pathway.

In this study we investigated the structure and function of antennal lobe projection neurons (PNs) in the fifth instar of the desert locust. The nymphal PNs did not show any structural difference in neither innervation nor projection compared to adult insects. The projection neurons did, however show a different response spectrum compared to adults; up to now, neither blend-specific PNs nor any PNs responding specifically to the major component in the adult blend, have been found. The physiological characteristics of PNs thus reflect behavioural differences between 5th instar nymphs and adults. PNs responded to both behaviourally active aggregation pheromone components, identified in the nymphal faeces, and to oviposition pheromone components. Obeng-Ofori *et al.*, 1994) identified two components in the nymphal faeces. In addition to these two components the nymphal antennal lobe interneurons also responded to a third component, veratrole, which is found both in the adult male faeces and in the oviposition pheromone. We expect that also veratrole is of behavioural significance, possibly connected to the aggregation behaviour.

06-042

CENTRAL NERVOUS PROCESSING OF AGGREGATION
PHEROMONES IN DESERT LOCUSTSS. Anton and B.S. Hansson
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Chemical cues are important for aggregation behaviour in the desert locust, *Schistocerca gregaria*. A number of aggregation pheromone components have been identified from the air surrounding adult male *S. gregaria*, and from the faeces of nymphs, whereas adult females seem not to produce aggregation pheromones. Adult male produced odours elicit aggregation behaviour only in adults, whereas nymphs respond only to nymphal volatiles (Torto *et al.*, Chem Ecol 20:1749-1762, 1994; Obeng-Ofori *et al.*, Entomol Exp Appl 73:85-91, 1994).

In this study we investigated the structure and function of antennal lobe interneurons, responding to single aggregation pheromone components and adult- and nymph-specific mixtures in adult males and females and in first instar nymphs of *S. gregaria*. The antennal lobe of adult *S. gregaria* contains over 1000 glomeruli which are arranged around a central fibre core. First instar nymphs have slightly fewer and smaller glomeruli. Single olfactory receptor neurons innervated several glomeruli within the antennal lobe. Projection neurons arborized in 10 to 25 glomeruli in adults and nymphs, and sent their axons to the protocerebrum. Projection neurons responded with different degrees of specificity to all behaviourally active aggregation pheromone components. Component-specific, blend-specific and generalist neurons were found in adults and first instar nymphs. In adults and nymphs most neurons responded to the adult male mix, but nymph mix-specific neurons were found too. The different behaviour of adults and nymphs is thus only partially reflected by the response characteristics of antennal lobe projection neurons.

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06-043

MODULATION OF LEG JOINT STRAND RECEPTORS OF
LOCUSTA MIGRATORIA BY BIOGENIC AMINES

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It has been shown in recent years that the responses of proprioceptors may be modulated by biogenic amines in various arthropod preparations. We are interested whether this also applies to insect strand receptors. Strand receptors are a special class of proprioceptive mechanoreceptors which are unusual because the somata of their sensory neurones are located in the central nervous system. In locusts, strand receptors are associated with appendages like antennae, mouthparts, and walking legs. In the legs there are four sense organs of this type associated with the subcoxal, the coxo-trochanteral, and the femoro-tibial joints.

Characteristically strand receptors span the joints in intimate association with power muscles. In the hind leg, for instance, the subcoxal receptor runs parallel to the anterior adductor muscle (M125), the two coxo-trochanteral receptors are embedded between the trochanteral levator muscles (M131, M132), and the strand of the femoro-tibial receptor runs parallel to the anterior accessory flexor muscle (M136b). Most, perhaps all these muscles are innervated by octopaminergic efferent DUM neurones. This agent might in principle reach the sensory dendrites of the strand receptor neurones by diffusion from synaptoid release sites in nearby power muscles. We therefore concentrate on studying possible effects of octopamine on strand receptor sensitivity, but other agents are being tested as well.

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06-044

OCTOPAMINE MODULATES THE NEUROMUSCULAR MOTOR
PATTERN ASSOCIATED WITH STRIDULATION IN THE CRICKET,
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Calling stridulation in the male cricket, *Gryllus bimaculatus*, consists of coherent, well defined chirps which are rigidly organized into discrete groups of three to five pulses. Calling stridulation was induced in the cricket by applying a constant DC-current to the brain. The stridulatory motor pattern associated with calling was then characterized by recordings made from the antagonistic opener (M99) and closer (M90) muscles driving the elytra. The neuromuscular motor pattern was characterized according to two parameters: 1) The phase relationship between M99 and M90 and 2) The underlying rhythm being generated within and between muscles M99 and M90.

The injection of octopamine into the abdominal haemocoel of a restrained cricket was undertaken when the cricket was calling. An analysis of the effects of octopamine on the stridulatory motor pattern indicated that the phase relationship appeared to be severely disrupted, due to an increase in M99 and M90 burst durations. However, the stridulatory cycle period from opener to opener burst was not significantly altered. The additional muscle activity observed between the chirps, represented by rapid short and soundless contractions of the elytra, did not cause any disruption to the underlying rhythm being generated by the antagonistic muscles. Therefore, octopamine was disrupting the neuromuscular activity associated with stridulation by causing additional spiking activity within and between the chirps. However, no disruption to the generation of the stridulatory motor pattern itself was observed. These excitatory effects could have resulted from the action of the octopamine on either the muscle or the CNS or both. These results, taken in combination, indicated that octopamine has a general excitatory effect of the neuronal elements driving the stridulatory motor neurons.

06-045

CONTRIBUTION OF THE CAUDAL NERVOUS SYSTEM TO THE GENERATION OF STRIDULATORY MOTOR PATTERNS IN CRICKETS

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The central nervous mechanism responsible for the genesis of stridulatory motor patterns is widely accepted to reside within the pro- and mesothoracic ganglia alone. However, we describe the experimental evidence which demonstrates that more caudal neuronal structures are intimately associated with the generation of calling and aggressive song patterns in the house cricket, *Acheta domesticus*, and the field cricket, *Gryllus bimaculatus*.

Bilateral transection of the ventral nerve cord, in freely mobile crickets, revealed that neural input from the metathoracic ganglion is a prerequisite for song production. Furthermore, transection of the ventral nerve cord (VNC) between the metathoracic ganglion complex and the second free abdominal ganglion resulted in severe temporal disruption to acoustic behaviour.

Acoustic and electromyographical recordings of stridulatory activity from restrained crickets during song production elicited by direct brain stimulation, subsequently confirmed these data. The intimate association between stridulatory and ventilatory rhythms may provide an explanation for the abolition of patterned motoneuronal output following VNC transection immediately anterior to the metathoracic ganglion. The existence of interneurons, believed to originate within the metathoracic ganglion, which co-ordinate and command the ventilatory and flight motoneurons in the thoracic ganglia has been suggested for the locust. It is possible that such cells are present in the crickets, and serve to drive the stridulatory central pattern generator directly.

An apparent role for structures located caudal to the metathoracic ganglion, is however, more surprising. It is suggested that the rhythmic centre for song production is distributed over a wide region of the thoracic and abdominal CNS, and that the anterior abdominal ganglia perform a secondary, but nevertheless important role during pattern genesis, serving to stabilise thoracic stridulatory rhythms.

06-047

OLFACTORY CONDITIONING OF THE PROBOSCIS EXTENSION REFLEX IN *SPODOPTERA LITTORALIS*

(Lepidoptera: Noctuidae)

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Most female moths use olfactory cues to find host plants for oviposition. The knowledge is limited whether the moths' ability to recognise different odours is acquired or genetic. Thus, the aim of the present study was to investigate the learning ability of a noctuid moth. The proboscis extension reflex (PER) was used as an olfactory conditioning model. The moths were trained to associate a conditioned stimulus (CS, geraniol 100µg applied onto a piece of filter paper) with an unconditioned stimulus (US, 40% sucrose solution). Four experiments were conducted: (1) The effect of CS/US interval +10, +5, +3, +1, -3, -5 and -10s (+, - indicated that the CS was presented before and after US respectively) was tested after one-trial learning. A conditioned response (CR, conditioned PER) was established at the interval +3 to +1. (2) The moths were trained with 0, 1, 2, 4, 6, 8 or 10 trials. After 1 trial learning, 46.7% of the moths responded to the CS, and the CR increased up to 76.6% after 10 trials. (3) Two groups of moths were given either sucrose solution or water as the US in 10 trials learning. The CR to the sucrose solution was 76.5%, whereas that to water was 23.3%. (4) The moths were trained with different intertrial intervals (ITI). After 10 trials learning, the CR was 46.7%, 80% and 56.7% at the ITI 1, 5 and 10 min respectively. The results suggest that the moths do have a capability to associate an odour with a sucrose solution, and that the ITI and the CS/US interval are important factors in the conditioning process.

06-046

EVIDENCE FOR THE ROLE OF THE ACCESSORY MEDULLA AS CIRCADIAN PACEMAKING CENTER IN THE BRAIN OF ORTHOPTEROID INSECTS

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Lesion studies have pointed to an area between the ventral medulla and the lobula as likely site of the circadian clock in cockroaches and crickets. This area is occupied by pigment-dispersing hormone-immunoreactive (PDH-ir) neurons, which innervate the accessory medulla (aMe), an appendage of the medulla. We investigated the organization of the aMe in locusts, crickets, and cockroaches, and present evidence for its possible role as a circadian pacemaker. In the cockroach *Leucophaea maderae* the aMe is not organized retinotopically. High concentrations of dense core vesicles in the aMe are matched by immunocytochemical localization of at least 7 partially colocalized neuropeptides. The aMe receives obviously dendritic as well as axonal innervation by PDH-ir neurons, which fulfill several morphological criteria for pacemaker candidates. Differences in the anatomy of the PDH-ir neurons may explain the differences in the coupling strength of the bilaterally paired pacemakers in crickets and cockroaches. After transection of both optic stalks the regeneration of PDH-ir processes to their original targets in the midbrain correlates with the regain of circadian locomotor activity. The intensity of PDH-immunostaining in the aMe shows a daily rhythm and injections of PDH into the optic lobe result in phase-dependent phase shifts of circadian wheel-running activity. Neurons of the aMe respond to photic stimuli and, in locusts are sensitive to polarized light. Taken together, these data support a role of the aMe as circadian pacemaking center in orthopteroïd insects. [Supported by DFG grants Ste 531 + Ho 950]

06-048

THE OLFACTO-CARDIAC RESPONSE AS A BIOLOGICAL MODEL FOR LEARNING AND MEMORY STUDIES

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In blowflies, olfactory stimuli can release the cardiac response of premature heart beat reversal, which has been described as a very sensitive indicator of sensory reception. Here we propose the paradigm of olfactory-induced cardiac response as a tool for testing the fly's ability to sense odours and learn and retain olfactory informations.

Adult *Protophormia terraenovae* blowflies were used. Electrophysiological recordings of heart activity (ECG) from the external body surface were performed on intact, restrained insects. Olfactory input was monitored (electroantennogram, EAG) simultaneously with ECG.

In learning tests, flies were trained to habituation by performing series of successive stimulations. Acquisition of habituation was measured as the number of effective stimulations needed to abolish the response. Memory formation was evaluated as the time required by flies trained to habituation in a first session of a stimulation series to show identical responsiveness in a second session of the stimulation series. Response dishabituation experiments were also performed and the dynamics of acquisition and retention of response dishabituation was determined.

06-049

DORSAL UNPAIRED MEDIAN NEURONES FORMING NEUROHAEMAL RELEASE SITES IN THE PERIPHERAL NERVOUS SYSTEM

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The efferent dorsal unpaired median (DUM) neurones represent a well-studied group of octopaminergic insect neuromodulatory cells. They are present in all ganglia of the central nervous system except the brain, they project bilaterally into the skeletal musculature, and have been shown to modulate neuromuscular transmission. The central branching pattern of numerous DUM neurones has been described in detail, but we know only very little about their peripheral branching patterns, and the target organs of individual DUM neurones are known in only a few cases.

One of the thoracic DUM neurones (DUM1B) innervates skeletal muscles, but also forms neurohaemal terminals on the surface of many peripheral nerves. DUM neurones forming such neurohaemal release sites are also found in the suboesophageal ganglion. They innervate antennal muscles but also the retrocerebral glandular complex. These results raised the question whether all efferent DUM neurones form such neurohaemal terminals in the periphery or whether this structural feature is unique for only a few such cells.

The results of a first survey (staining the well known DUM345, DUMETi and DUMDL neurones) indicate that neurohaemal ramifications appear to be a speciality of only a few DUM neurones. The thoracic DUM345 neurones establish a small neurohaemal area in only one particular peripheral location. No neurohaemal terminals were observed after staining DUMETi or DUMDL.

06-050

PILOCARPINE-INDUCED FEEDING MOTOR RHYTHMS OF THE ISOLATED SUBOESOPHAGEAL GANGLION OF *LOCUSTA MIGRATORIA*

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The muscarinic agonist pilocarpine has been shown to induce motor rhythms resembling those observed during locomotion in locusts and stick insects. We applied pilocarpine to the isolated suboesophageal ganglion (SOG) in order to find out whether this resulted in motor output similar to that observed in intact locusts during feeding. Our ultimate goal is to develop a preparation that allows us to study the interactions between neurones generating the feeding motor rhythm with intracellular methods, since this approach is impossible in intact insects because of the inaccessibility of the SOG.

Simultaneous recordings of mandibular opener and closer nerves on both sides show that pilocarpine (10^{-5} - $5 \cdot 10^{-4}$ M in the bath) elicits motor rhythms comparable to that observed in myogram recordings of the same muscles in intact feeding locusts: Opener units in both left and right nerves fire synchronously, but out of phase to the synchronously firing units in both left and right closer nerves. These rhythmic discharges during "fictive feeding" show much variability when compared to the motor rhythms observed in intact locusts. When comparing burst frequency and duration, however, rhythmic motor activity produced by the isolated ganglion often shows surprising similarities with the naturally occurring rhythms.

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06-051

DIFFERENTIALLY EXPRESSED GENES IN THE CENTRAL NERVOUS SYSTEM OF *LOCUSTA MIGRATORIA* AND *SCHISTOCERCA GREGARIA*

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The areas of the insect central nervous system (CNS; e.g. optic lobes, antennal lobes, mushroom bodies, thoracic ganglia) have different functions in information processing. This is based in part on the different molecular equipment of their neurons, e.g. within the optic lobes other receptor molecules are expressed than within the mushroom bodies. On the other side, also in single neurons signal cascades are no rigid processes. They can be modulated on every step, and thus be adapted to the respective requirements. Such modulations can have different time constants by which they are subdivided into short-term or long-term modulations. Both cases of functional adaptation, either the brain area specific or the long-term modulatory, are based on differentially expressed genes.

We investigate these differential gene expressions in the CNS of migratory (*Locusta migratoria*) and desert locusts (*Schistocerca gregaria*). In the first case mRNA is prepared from different areas of the CNS, and the reverse transcribed cDNA is analysed by the Differential Display-PCR (DD-PCR; Liang and Pardee, Science 257, 967-971, 1992). In the second case genes are activated by second-messenger analogous, and expressed in neurons from primary cultures. The cDNA produced from these cells is also analysed by the DD-PCR.

The results of these investigations show: (1) There is an area specific gene expression as well in the optic lobes, as in the dorsal protocerebrum with the mushroom bodies, or within the thoracic ganglia, where we find specific cDNA populations. (2) The expression pattern of the neurons from the primary cultures is changed in time, e.g. genes with different time constants are activated. Some genes are active only about 30 min after start of the experiment, others after about 4 h. This may have relevance to long-term modulation.

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06-052

EFFECTS OF 20-HYDROXYECDYSONE AND DRUGS ON NEURONAL CELL DEATH IN THE CENTRAL NERVOUS SYSTEM OF *BOMBYX MORI* (LEPIDOPTERA)M. Kim, H. R. Kim¹

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In holometabolous insects, dramatic changes in external morphology from crawling larvae to flying reproductive adults are accompanied with changes in internal organs such as central nervous system and muscles. Especially the central nervous system undergoes neurometamorphosis involving neurogenesis, respecification of neuronal connectivity, and cell death. In this study, total numbers of neuron from last instar larvae to adults in *B. mori* abdominal ganglion (A3) were investigated to know the period of cell death. Two periods of cell death were present; the larva-pupal transition and the pupal-adult transition. To eliminate the ecdysteroid-secreting prothoracic glands in the thorax and interrupt descending neural pathway from the brain and thoracic ganglia, abdominal isolation was conducted. Neuronal cell death on abdominal ganglion (A3) was significantly delayed. To study the interaction between cell death and 20-hydroxyecdysone (20-HE), 20-HE was injected into lateral part of thorax every 12 hours for three days. To investigate this cell death is an active process, I tested the effects of RNA and protein synthesis inhibitor (Actinomycin D and Cycloheximide) on the survival of neurons.

06-053

IN VITRO PHOSPHORYLATION OF NERVOUS SYSTEM PROTEINS OF *BOMBYX MORI* DURING LARVAL-PUPAL-ADULT TRANSFORMATION: INVOLVEMENT OF Ca^{2+} /CALMODULIN DEPENDENT KINASE II AND JUVENILE HORMONE

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Protein phosphorylation is a post-translational modification that alters protein function and its interactions with other proteins, and is an important mechanism controlling nervous system function and development. *In vitro* phosphorylation of CNS (Central Nervous System) extracts from larval, pupal and adult *Bombyx mori* were carried out in the presence and absence of calcium and calmodulin. Polyacrylamide gel electrophoretic analysis followed by autoradiography revealed the phosphorylation of several proteins of which a 47 and 115 kDa polypeptides were the major phosphoproteins labelled with [^{32}P] in the absence of calcium. When the phosphorylation was performed in the presence of $CaCl_2$ (1 mM) there was a striking difference in the phosphorylation pattern of CNS proteins, two new polypeptides with molecular weights of 56 and 45 kDa were phosphorylated and there was an enhanced phosphorylation of the 115 kDa protein. Further 1 μ M calmodulin stimulated the phosphorylation of 56 and 45 kDa proteins by several fold.

Studies using the inhibitors of calcium dependent protein kinases revealed that KN-62, a specific inhibitor of Ca^{2+} /calmodulin-dependent protein kinase II inhibited the phosphorylation of the 56 and 45 kDa polypeptides by about 70%. Using calmodulin gel overlay and two dimensional gel electrophoresis we have putatively identified the phosphorylation of 56 kDa protein as Ca^{2+} /calmodulin kinase II autophosphorylation. *In vitro* culture of larval CNS in presence of JH I significantly stimulates the phosphorylation of 47 kDa protein. Degree of phosphorylation of the major phosphoproteins shows a distinct developmental profile. Further studies on the identification and characterization of these proteins may provide insight into their physiological function during neurometamorphosis.

06-055

DEVELOPMENTAL CHANGES OF LOCUSTATACHYKININ-1-IMMUNOREACTIVE NEURONS IN POSTEMBRYONIC CENTRAL NERVOUS SYSTEMS OF THE COMMON CUTWORM, *SPODOPTERA LITURA*

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Antiserum raised against the neuropeptide locustatachykinin-1 (LomTK-1) were used to investigate developmental changes in LomTK-1 containing neuron in postembryonic central nervous systems of the common cutworm, *Spodoptera litura*. In the brain of 1st instar larva, 4 pairs of locustatachykinin-1-immunoreactive(LomTK-IR) neurons was detected. In the developmental stages from the 1st instar larva to the 5th instar larva, the number of LomTK-1-IR neurons gradually increases, especially to 45 pairs. During the metamorphic changes from prepupa to pupa, the number of LomTK-1-IR neurons gradually decreased from 21 pairs to 12 pairs, and then abruptly increases to about 45 pairs in the 7-days-old pupa and the adult. The LomTK-1-IR neurons in all the developing brains supply their nerve processes to most brain neuropils, and 2 pairs of LomTK-1-IR nerve fibers are running into all the ventral ganglia. The suboesophageal ganglion, which has been separated from brain, is incorporated to the brain during development from prepupa to 1-day-old pupa, thereby forming cerebro-suboesophageal complex. The 2nd thoracic ganglion in the 1st instar larva has 1 pair of LomTK-1-IR neurons, while 2nd and 3rd thoracic ganglia of 2nd instar larva contain 1 pair and 3 LomTK-1-IR neurons, respectively. However, LomTK-1-IR neurons are included in all the ventral ganglia from the 3rd instar larva to the adult. In each ventral ganglion, the number of LomTK-1-IR neurons gradually increase from the 1st instar larva to the 5th instar larvae, and then decrease from the 6th instar larva to the adult. All the ventral ganglia contain 2 pairs of LomTK-1-IR nerve fibers which are running from the brain to the terminal abdominal ganglia.

06-054

IMMUNOCYTOCHEMICAL MAPPING OF SEROTONERGIC NEURONS IN CENTRAL NERVOUS SYSTEMS OF THE COMMON CUTWORM, *SPODOPTERA LITURA* DURING POSTEMBRYONIC DEVELOPMENT

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This study has been carried out to investigate the postembryonic development of neural network of serotonin-immunoreactive(5-HTi) neurons in the brain and ventral ganglia of the common cutworm, *Spodoptera litura*. The brains of 3rd instar and 4th instar larvae contain about 26 pairs of 5-HTi neurons, which are located in medial and marginal regions of them. The 6th instar larval brain includes about 24 pairs of 5-HTi neurons which are evenly distributed in it. The 5-HTi nerve processes are mainly intrinsic to the brains. 5-HTi neurons are included as about 14 pairs in suboesophageal ganglion, as about 11 pairs in three thoracic ganglia, and two to five 5-HTi neurons in each abdominal ganglia, in which the number and distribution pattern of 5-HTi neurons and nerve processes are very similar without regard to developing larval stages. In all the larval stages, a pairs of 5-HTi nerve fibers, which run down in parallel, project from the brain to the terminal ganglion. The brains, which have been developed into pupal stage, have less 5-HTi neurons(about 33 pairs) in early pupal stage. The 5-HTi nerve fibers has been also reduced in number. They form about two 5-HTi commissural nerve fiber bundles, which are innervated into contralateral cerebral neuropils, show rich arborizations. In adult, 5-HTi neurons abruptly increase in number to about 83 pairs. The 5-HTi nerve processes are also mostly intrinsic to brain. But some of them are extrinsic to the ventral ganglia.

06-056

POSTEMBRYONIC DEVELOPMENT OF LEUCOKININ-1-IMMUNOREACTIVE NEURONS IN CENTRAL NERVOUS SYSTEMS OF THE COMMON CUTWORM, *SPODOPTERA LITURA*

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The developmental changes of leucokinin-1-immunoreactive(LK-1-IR) neurons in the postembryonic central nervous systems(CNS) of the common cutworm, *Spodoptera litura*, were investigated by using antiserum that was raised against leucokinin-1(LK-1). The whole-mount immunocyto- chemistry in developing CNS has demonstrated that LK-1-IR neurons exist as 2 pairs in the brain of 2nd instar larva, and then gradually increase in number to 16 pairs in the brain of 5th instar larva. The LK-1-IR nerve processes found from the 2nd instar larva to the 5th instar larva are mostly intrinsic to the brain. However, 2 pairs of LK-1-IR nerve fibers run through all the ventral ganglia. While the brain of the 6th instar larva has 13 pairs of LK-1-IR neurons, the brains of prepupa, 1-day-old, 3-days-old, 5-days-old, and 7-days-old pupae contain just 2 or 3 pairs of LK-1-IR neurons. The adult brain has 6 pairs of LK-1-IR neurons. After the pupation, the LK-1-IR nerve processes abruptly decrease, due to the reduction in number of LK-1-IR neurons. In case of the ventral ganglia, 1st instar larva possesses 2 pairs of LK-1-IR neurons in the 3rd thoracic ganglion and 1 pair each in 3rd, 4th, 5th and 6th abdominal and terminal ganglia. In 2nd and 3rd instar larvae, additionally 2 pairs and 3 pairs of neurons appear in abdominal ganglia, respectively. The number and localization of the LK-1-IR neurons is very similar from the 3rd instar larva to the prepupa. There is no in the larval stages. During metamorphosis from the prepupa to the adult, the number and distribution of the LK-1-IR neurons showed similar patterns in all ventral ganglia. The LK-1-IR nerve processes in the ventral ganglia of all postembryonic developmental stages are mostly projecting to neurohemal organs, but intrinsic nerve fibers are also found.

06-057

ACCUMULATION OF PEPTIDE NEUROHORMONES IN PARASITIZED CATERpillARS: IDENTIFICATION OF FLRFamide PRECURSORS

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We have shown that parasitization of *Manduca sexta* by the braconid wasp *Cotesia congregata* leads to an accumulation of immunoreactive peptides, both in the endocrine cells of the midgut and in neurosecretory cells of the CNS. This raises the possibility that one consequence of parasitization is a general block in release of peptide hormones, including those associated with development. We have exploited this accumulation to isolate and sequence two new FLRFamides from the midguts of parasitized *M. sexta*. These peptides, 24 and 39 amino acids in length (F24, F39), have the sequence KRQDVVHSFLRFamide at their C-termini; thus, they have the characteristics expected of a pro-hormone for a peptide (pEDVVHSFLRFamide) previously identified from the CNS. Preliminary data suggest that F24 and F39 are also present in the CNS, thus supporting their presumptive roles as prohormones. Interestingly, the decapeptide was not found in the midgut, suggesting that F24 and F39 themselves may function as endocrines or paracrine from the midgut. Earlier studies showed that the decapeptide could be distinguished in its action on visceral muscle from two heptapeptide FLRFamides (GNSFLRFamide, DPSFLRFamide) also isolated from *M. sexta* CNS. To determine whether a structural basis exists for these findings, we have used two-dimensional nuclear magnetic resonance to investigate the secondary structures of the *M. sexta* FLRFamides. We find that the heptapeptides do not exhibit secondary structure in solution, but that the decapeptide does, forming a γ -turn about His⁵-Phe⁷.

06-058

NEURAL INACTIVATION OF SEX PHEROMONE PRODUCTION AFTER MATING IN THE SILKWORM MOTH, *BOMBYX MORI*

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The production of sex pheromone in the moth is under the control of a pheromonotropic factor, pheromone biosynthesis-activating neuropeptide (PBAN). Neurosecretory cells with immunoreactive PBAN have a soma in the subesophageal ganglion (SG) and project an axon to the corpus cardiacum (CC) via a branch of the maxillary nerve.

A virgin female of the moth has a large amount of sex pheromone in the pheromone gland. After mating, pheromone titer declined with a time course closely resembling that of decapitated females. The inactivation of pheromone production after mating was prevented when the ventral nerve cord was transected before mating. In contrast, the post-mating inactivation of pheromone production was not prevented when the ventral nerve cord was cut 1 h after the initiation of mating. Although females produced only a small amount of pheromone when the connection between the brain and the SG was cut at an early pupal stage, mating did not induce a significant decline of pheromone production in such females. When connection between both hemispheres of the brain of a virgin female was cut, pheromone titer declined to near zero without mating.

The results suggest that (1) mating may induce a complete shut down of the release of the pheromonotropic factor from the CC at an early stage of copulation, (2) the brain has a neural mechanism that activates the putative PBAN producing cells in the virgin female, (3) inactivation of pheromone production after mating is mediated by a neural signal that travels up the ventral nerve cord to the brain-SG complex to switch off the neural mechanism.

06-059

BRAIN FACTORS CONTROLLING DIAPAUSE HORMONE RELEASE FROM THE SUBESOPHAGEAL GANGLION OF THE SILKWORM, *BOMBYX MORI*

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Bombyx embryonic development is arrested at the late gastrula stage in response to the peptidic diapause hormone (DH) secreted from the subesophageal ganglion (SG) in young pupae of diapause egg producers. DH acts upon the oocytes of diapause egg producers and alters their developmental program. Although DH's amino acid sequence and gene structure were determined, the regulation of DH secretion in pupal brains has remained a mystery for a long time.

Free nucleotides in pupal brains were examined in a bivoltine race (Daizo), using high pressure liquid chromatography (HPLC). Concentrations were nearly the same level as observed in rat brains. Major differences in monophosphates, diphosphates and triphosphates of purine and pyrimidine nucleotides, and an unidentified nucleotide derivative were observed between silkworms which were exposed to regimens of continuous light and warm conditions (27°C) and those exposed to continuous darkness and cold conditions (15°C) throughout maternal embryonic development. In particular, concentrations of ADP, ATP, GDP, GTP, IDP, ITP, UDP and UTP, were more abundant in the pupal brains of insects exposed to continuous light and warm conditions. When brain-subesophageal ganglion complexes from non-diapause egg producers were cultured with mixtures of nucleotides, the resulting culture medium was highly effective in eliciting diapause in non-diapause egg producers.

On the other hand, using capillary SDS gel electrophoresis of pupal brain extracts, we found significant differences between diapause egg and non-diapause egg producers. The brain extract of diapause egg producers also induced *in vitro* secretion of a diapause hormone-like material from the brain-subesophageal ganglion complexes of the non-diapause egg producers. These results suggest that the phosphorylating enzymes of brain nucleotides and the brain peptides have a role in diapause induction.

06-060

LOCALIZATION OF ALLATOTROPIN IN THE BRAIN AND GANGLIA OF THE HAWKMOOTH, *AGRIUS CONVULVULI*

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Allatotropin (AT) is a neurosecretory peptide that stimulates juvenile hormone secretion in the corpora allata of the insect. However, AT appears to be active only in adult Lepidoptera and has no effect on larvae or on species in other orders. Therefore, to clarify the role of AT, the localization of AT peptides and gene transcripts in the nervous system of the hawkmoth, *Agrius convolvuli*, were investigated by immuno-histochemistry and *in situ* hybridization.

Immunohistochemical investigations with a monoclonal antibody against synthetic AT revealed that AT peptides were present in many cells of the brain and the frontal, subesophageal and prothoracic ganglia.

DIG-labeled DNAs and RNAs were synthesized from the AT cDNA and used as hybridization probes. After *in situ* hybridization on whole mount and sectioned brain samples, several cells were positively labelled.

06-061

CARBACHOL-INDUCED PROTEIN PHOSPHORYLATION IN THE BRAIN-CORPUS CARDIACUM- CORPUS ALLATUM COMPLEX OF THE SILKWORM, BOMBYX MORI.

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As to the control of PTTH release, we have so far found that carbachol (acetylcholine agonist) induces PTTH release via the muscarinic acetylcholine receptor, and that protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase (Ca²⁺/CaM PK) are involved in carbachol-induced signal transduction. These findings suggest that protein phosphorylation is important for PTTH release. Accordingly, carbachol-induced protein phosphorylation in the brain-corpus cardiacum-corpus allatum complex, in parallel to carbachol-induced PTTH release, was examined.

Phosphorylations of approximately 32 kD, 25 kD and 21 kD proteins were depended on the carbachol concentrations in the range of 10 μM - 1 mM, and occurred within 5 min. The time- and dose-dependency of these phosphorylations were consistent with those of the carbachol-induced PTTH release. Furthermore, the 25 kD protein seemed to be phosphorylated by PKC, while Ca²⁺/CaM PK might phosphorylate the 21 kD protein.

Consequently, the good correlation between the carbachol-induced protein phosphorylations and the carbachol-induced PTTH release suggest that the 25 kD and 21 kD phosphorylated proteins, at least, should play important role in carbachol-induced PTTH release. We are exploring the function of phosphorylated proteins in PTTH release.

06-063

IMMUNOCYTOCHEMISTRY OF A NOVEL INSECT GABA RECEPTOR SUBUNIT

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Following our recent cloning of a novel γ-aminobutyric acid (GABA) receptor subunit gene *Resistance to dieldrin* or *Rdl* from the cyclodiene resistance locus in *Drosophila melanogaster*, we were interested in defining its pattern of expression during development. Here we report the raising of an anti-*Rdl* polyclonal antibody that recognizes a single protein of the expected 65 kDa size in immunoblots of *Drosophila* head homogenates. *In situ* hybridization using *Rdl* cDNA probes and the anti-*Rdl* antibody shows that *Rdl* message and protein are expressed globally in the developing central nervous system (CNS) of 15-17 hr embryos. No message can be observed on or before 12-13 hr. Interestingly, despite the use of GABA in both the peripheral and CNS of insects, *Rdl* GABA receptor subunits appear to be confined to the CNS. Detailed immunocytochemistry of *Drosophila* brain sections showed particularly strong anti-*Rdl* antibody staining in the optic lobes, ellipsoid body, fan shaped body, ventrolateral protocerebrum and the glomeruli of the antennal lobes. Results are compared with the distribution of staining observed in the insect CNS with antibodies against GABA itself, synaptotagmin (a synaptic vesicle protein) and a second *Drosophila* subunit which appears to be a homolog of the vertebrate GABA_A receptor β subclass.

06-062

LIGAND-SPECIFIC COUPLING OF A NOVEL CLONED DOPAMINE RECEPTOR FROM *DROSOPHILA MELANOGASTER* WHEN EXPRESSED IN *XENOPUS* OOCYTES.

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In insects dopamine receptor subtypes may be involved in memory and learning. Dopamine receptors belong to the G-protein coupled receptor superfamily of seven transmembrane domain proteins. Five distinct classes of dopamine receptor have been identified by cloning in mammals. These fall into two broad pharmacological categories D1-like and D2-like. A D1-like receptor affecting adenylyl cyclase activity has already been identified in *Drosophila* (Gotzes et al., 1994; Sugamori et al., 1995). We have recently cloned a novel G-protein coupled dopamine receptor (DopR99B), expressed in *Drosophila* heads, which may define a novel class of dopamine receptors.

We report here that when expressed in *Xenopus* oocytes the activated DopR99B increases intracellular Ca²⁺ levels and also increases cyclic AMP levels. The rank order of potency for biogenic amines in stimulating both second messenger effects is dopamine > norepinephrine > epinephrine > tyramine. Octopamine and 5-hydroxytryptamine are not active at concentrations up to 100 μM. The pharmacological profile for synthetic antagonists on both second messenger responses was identical and suggested that DopR99B is a D1-like dopamine receptor. The pharmacological profile for synthetic agonists again suggests a D1-like dopamine receptor, but was different for the two second messenger systems assayed.

Thus, DopR99B exhibits ligand-specific coupling to different second messenger pathways, a phenomenon exhibited by a range of other aminergic and peptidergic G-protein coupled receptors from both invertebrates and vertebrates (Robb et al 1994; Evans et al 1995). This suggests that the receptor exhibits a different agonist profile for synthetic ligands depending upon which second messenger systems are assayed.

06-064

NEUROTRANSMITTER TRANSPORTERS IN INSECTS: ISOLATION, CHARACTERIZATION AND PHARMACOLOGY OF THE γ-AMINO BUTYRIC ACID (GABA) TRANSPORTER IN *MANDUCA SEXTA*.

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Synaptic transmission termination occurs by several mechanisms including the uptake of the neurotransmitter molecules into the presynaptic neuron by specialized plasma membrane transport proteins. We have cloned a (DABA)-sensitive GABA transporter and a novel transporter from a cDNA library from *Manduca sexta* embryo. The GABA transporter cDNA clone, MasGAT, has high sequence homology to known mammalian GABA transporters. The MasGAT transcript is about 5.5kb with an open reading frame (ORF) of 1793bp, while that of the novel transporter is 4.9kb with an ORF of 2181bp. Injection of a 2.2kb cRNA from this clone into *Xenopus* oocytes results in ³H-GABA transport, while the transport of the novel transporter is under investigation. Michaelis-Menten kinetic analysis shows that GABA transport occurs by a high affinity and saturable process suggesting that it is carrier-mediated. Ion substitution studies also show the transport process to be highly dependent on extracellular Na⁺ gradient, a finding that is consistent with properties of known mammalian neurotransmitter transporters. Although MasGAT shares certain pharmacological similarities with known mammalian GABA transporters this transporter has pharmacologically distinct from the known mammalian GABA transporters.

Section 7

Insect Immunity

07-002

IMMUNE RECOGNITION AND REACTIVITY IN *DROSOPHILA*

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Although various cellular and biochemical effector responses have been well documented for many insects, comparatively little is known of the molecular mechanisms underlying the prefatory non-self recognition response, or of the nature, specificity and mode of action of the killing molecules. The *Drosophila*-wasp parasitoid system provides unique opportunities to examine the interactions between immune competent and susceptible host strains with resistant and susceptible strains and species of parasitoids. Immune reactivity in this host is manifested in part by the proliferation, differentiation, mobilization, aggregation and adhesion of hemocytes to form multilayered capsules around parasitoids. Eumelanin forms on the surface of the foreign object and among the hemocytes comprising the innermost layers of the capsule. Immune capacity in *Drosophila* infested by the wasp *Leptopilina boulardi* is due to a single dominant allele located on the second chromosome. This trait is strain and species specific, with separate alleles encoding recognition of different parasitoid species. Immune capacity is severely compromised in mutants deficient in dopa decarboxylase, suggesting the pathway involving the synthesis and oxidation of dopamine is essential for killing parasitoids. Implicated as cytotoxic molecules in *Drosophila* cellular immunity are quinoid precursors of eumelanin and/or certain reactive oxygen species generated during melanogenesis. The identity of the non-self recognition mechanism(s) triggering the effector responses has not been established. What appears to be essential for a non-self recognition process is a system of communication among hemocytes that potentiates their collaborative interactions with foreign entities. Non-self discrimination and collaborative hemocytic interactions with parasitoids may involve cell adhesion molecules (e.g., integrins, laminins, proteoglycans), regulatory polypeptides (cytokines), and/or agglutinins (lectins).

07-001

RECOGNITION OF NON-SELF IN INSECT IMMUNITY - THE RELATIVE ROLES OF HAEMOLYMPH LECTINS AND COMPONENTS OF THE PROPHENOLOXIDASE CASCADE

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Recent experiments indicate, unequivocally, that haemolymph lectins of insects function in the recognition and clearance of foreign invaders from the circulation, as well as important determinants of parasite colonization of vector species. Work in the cockroach, *Blaberus discoidalis*, has shown the presence of multiple lectins with differing specificities capable of recognising a range of microorganisms. Purification, characterisation and sequencing of these molecules has revealed structural and functional similarities to the mannose-binding proteins of vertebrates, which activate complement during innate immunity in these animals. Apart from the lectins, other factors generated during activation of the prophenoloxidase in insects also appear to function in the identification of non-self and mediation of the cellular defences.

The evidence for the relative roles of the lectins and of components of the prophenoloxidase cascade in insect immunity is discussed and a model for their possible interaction in the cellular defence reactions is presented.

07-003

THE PURIFICATION AND POSSIBLE ROLE OF TWO LECTINS ISOLATED FROM *RHODNIUS PROLIXUS* - THE VECTOR OF SOUTH AMERICAN TRYPANOSOMIASIS (CHAGAS' DISEASE)

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Lectins are known to have a wide range of important biological functions. Recently, several studies in haematophagous insect vectors, such as tsetse flies and blackflies, have shown that lectins, present in the midgut and/or haemolymph of the insect, may play a part in the establishment of parasitic infections in the host.

In this paper, we present the results of the purification and characterisation of a crop agglutinin and a haemolymph lectin isolated from *Rhodnius prolixus*, the vector of South American trypanosomes. We offer preliminary *in vivo* and *in vitro* evidence to suggest that the purified lectins may be implicated in the development and maturation of these trypanosomes.

07-004

IDENTIFICATION OF INDUCIBLE SERINE PROTEASES OF *SIMULIUM DAMNOSUM* S.L. (DIPTERA: SIMULIIDAE) FOLLOWING AN *ONCHOCERCA OCHENGI* (NEMATODA: FLIARIOIDEA) INFECTION USING TARGETED DIFFERENTIAL DISPLAY

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One of the earliest visible changes in the haemolymph of *Onchocerca* infected *S. damnosum* s.l., the most important vector of human and bovine onchocerciasis in West-Africa, is the appearance of phenoloxidase. As in other insects it could be shown that this enzyme has to be activated by serine proteases. In order to identify inducible proteases in infected *S. damnosum* s.l. we used Differential Display Reverse Transcription PCR (DDRT-PCR) using homology based generic primers. So far three serine protease have been clearly identified, one of which represents a trypsin like molecule. Based on recent sequence information of the cleavage site of prophenoloxidase from other arthropods this type of protease is likely to be involved in the cleavage of the pro-enzyme. At the moment we are in the process of characterizing the cleavage site of *S. damnosum* s.l. prophenoloxidase using 5' RACE-PCR following the successful cloning of prophenoloxidase-like DDRT-PCR-derived products to prove whether or not this is also the case for *S. damnosum* s.l. In the future it is envisaged to use recombinant active serine proteases for *in vitro* and *in vivo* tests to study the activation of prophenoloxidase in *S. damnosum* s.l.

07-006

REGULATORY AND FUNCTIONAL ASPECTS ON HEMOLIN, AN INSECT IMMUNE PROTEIN OF THE IMMUNOGLOBULIN SUPERFAMILY (IgSF)

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Hemolin was first identified in the giant silkworm, *Hyalophora cecropia*, as an immunoglobulin-related molecule involved in immune response. It is present at low levels in the naive insect, and strongly upregulated by bacterial infection, lipopolysaccharides (LPS) or phorbol ester (PMA), and also slightly by wounding.

By UV-cross linking experiments, hemolin was shown to bind to *E. coli* LPS. This binding could be competed by Lipid A, indicating its specificity of binding to Gram-negative bacteria. Pure hemolin inhibits hemocyte aggregation, a finding that can be explained by the recently identified membrane form of hemolin and by the homophilic binding properties of soluble hemolin. Moreover, hemolin was shown to enhance phagocytosis of yeast by both hemocytes and *Drosophila* mbn-2 cells. The phagocytic activity was further increased by addition of LPS. This activity was correlated with increased protein kinase C phosphorylation, which in turn could be inhibited by PKC inhibitors.

The *Hemolin* gene organization shares features with cell adhesion molecules of the IgSF. κB-motifs are found in one of the introns as well as in the upstream region. These regions did however not confer LPS induction in transfection assays.

07-005

RECOGNITION OF NON-SELF IN INSECT IMMUNITY - MODULATION OF IMMUNE REACTIONS BY OPSONIZATION

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Mechanisms responsible for opsonic effects have been studied in our laboratory. Phagocytosis of FITC-labelled yeast by isolated plasmatocytes (PL) from *Galleria mellonella* larvae significantly increased after preincubation of yeast cells in cell free haemolymph (= plasma). The portion of phagocytically active PL was enhanced from about 5% to 30-40%. Yeast cells opsonized in plasma from *G. mellonella* were phagocytosed at higher rates also by haemocytes from other arthropods (*Agrotis segetum*, *Zophobas adactylus*, *Astacus astacus*) and by so-called "Gäuff cells", a blood tumor cell line from *Drosophila melanogaster*, indicating a certain universality of the haemocyte-opsonin interaction.

Several molecules acting as opsonins in the haemolymph of *G. mellonella* have been recognized. One of these was found to be Apolipoprotein III (ApoLpIII), another proved to be a component of the prophenoloxidase activating system and one is a prostaglandin-like molecule. RGD-dependent cell adhesion of ApoLpIII has been demonstrated (Wiesner et al., submitted), it is also known that such cell adhesion proteins function in crustaceans (Thörnqvist et al., 1994). In addition to its opsonic effect, ApoLpIII acts also as a stimulator of cell activity and induces neosynthesis of antimicrobial peptides.

Granular cells (GR) represent the most probable source for opsonic material because this type of blood cell reacts by exocytosis upon contact with foreign substrates and extrudes material which sticks to the foreign surfaces. Monoclonal antibodies specific to GR have been prepared to stain such material and to block its opsonic capacity. Possible mechanisms of immune modulation by opsonization are discussed.

07-007

DETECTION AND CHARACTERIZATION OF AN INDUCIBLE METALLO-PROTEASE-INHIBITOR IN *GALLERIA MELLONELLA*

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Protease inhibitors are presumed to play an important role in inhibiting undesired proteolytic activity of enzymes released by invading pathogens. Several protease inhibitors have been described in hemolymph and cuticle from different insects, but an inhibitory activity against metallo-proteases was never reported before. Insect pathogens e.g. the entomogenous fungi *Metarhizium anisopliae* and *Beauveria bassiana* are known to produce metallo-proteases among other proteolytic enzymes during infection. Within the hemolymph of the Greater Wax Moth *Galleria mellonella* we demonstrated for the first time the occurrence of an metallo-protease-inhibitor in insects. It strongly inhibited thermolysin but not trypsin or elastase activity. In contrast to trypsin- or elastase inhibitory activity thermolysin inhibition was only detectable after intrahemocoelomic injection of provokers like zymosan, yeast cells or *M. anisopliae* and *B. bassiana* blastospores. The time dependant increase of metallo-protease-inhibitory activity in cell free hemolymph after injection was correlated with the lysozyme and antibacterial activity measured with inhibition zone assays, suggesting the release of an inhibitor during humoral immune response. For the detection and characterization of the metallo-protease-inhibitor we used a modified azocoll-assay and thermolysin as a sorbent for purification. The metallo-protease-inhibitor was resistant to heat treatment up to 100°C and to trichloric acid precipitation and it was not retained by ion exchange- or blue sepharose-columns.

07-008

CELL-CELL COOPERATION IN RECOGNITION AND ENCAPSULATION OF FOREIGN TARGETS BY INSECT HEMOCYTES

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Haemocytes play an essential role in defending insects against pathogens and parasites that enter the haemocoel. A primary defense response is encapsulation; a process in which haemocytes attach to the foreign organism and kill it. Whether encapsulation requires cooperation between specific subpopulations of haemocytes is unknown. Using monoclonal antibodies specific to different subpopulations of haemocytes, we investigated the process of capsule formation in the moth *Pseudoplusia includens*. Immunocytochemical staining demonstrated that capsule formation began when granular cells attached to the foreign target. This was followed by attachment of multiple layers of plasmatocytes. Termination of capsule formation occurred when a subpopulation of granular cells formed a monolayer around the periphery of the capsule. In vitro encapsulation assays revealed that neither granular cells nor plasmatocytes were capable of forming a capsule independently. However, plasmatocytes would encapsulate targets if granular cells were present or if targets were preincubated in medium conditioned by granular cells. Biochemical mediators involved in recognition of foreign targets and cell-cell adhesion during capsule formation are discussed.

07-009

PROTEINASE INHIBITORS OF THE MOSQUITO *Aedes aegypti*, PART OF THE IMMUNE SYSTEM?

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In *Aedes aegypti*, proteinase inhibitors are present in all developmental instars and are even enhanced in blood-fed females. This led to the assumption of suppressed chymotryptic activity during blood digestion. In blood-fed females, inhibitor activity against chymotrypsin was twice that of sugar-fed females. This inhibitory activity was localized mainly in the fat body, some of it in maturing oocytes, and was only marginal in the midgut.

Aedes aegypti chymotrypsin inhibitor (AACI) was isolated and purified by HPLC, revealing an extremely thermo- and pH-stable peptide with a molecular mass of 5.75 kD, established by mass spectrometry.

AACI activity was active against proteinases of different mosquito species as well as against bovine α -chymotrypsin and trypsin, but not against subtilisin, pancreatic elastase, nor fungal proteases. AACI did not interfere with bacterial growth.

The amino acid sequence of AACI showed 50-55% identity with small serine proteinase inhibitors from *Locusta migratoria*, which are known to be involved in the regulation of the prophenoloxidase (PPO) activation cascade.

In conclusion, the hypothesis of AACI as a regulator of chymotrypsin during blood digestion is abandoned; instead we assume a functional role in the mosquito immune system.

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07-010

ANTIMICROBIAL PEPTIDES IN INSECTS : A BIOCHEMICAL AND PHYLOGENETIC STUDY.

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The immune defense of insects against microorganisms involves cellular and humoral effector mechanisms. The humoral defense facet is characterized by the rapid appearance in the hemolymph of a battery of potent antimicrobial substances. These molecules are synthesized in the fat body in response to a microbial challenge and released into the blood where their respective concentrations range between 1 μ M and 100 μ M. Since the characterization in 1981 by Boman and associates of the cecropins, more than 100 structures have been reported. We have isolated from various insect species over 60 of these molecules. They are small cationic peptides which can be classified into four distinct families : cecropins (linear molecules without cysteines forming amphipathic helices), cyclic peptides (peptides with one to four intramolecular disulfide bridges), proline-rich peptides (with or without an O-glycosylated substitution) and glycine-rich peptides. Among the molecules which we have characterized, four are of particular interest. (1) Drosocin, which has been isolated from *Drosophila melanogaster*, is the first antibacterial peptide carrying an O-glycosylated substitution. It is the prototype of a new class of insect antibacterial peptides and this type of post-translational modification is observed in other molecules. (2) Drosomycin, isolated from *Drosophila*, is a cysteine-rich peptide with four intramolecular disulfide bridges. This molecule kills filamentous phytopathogenic and human pathogenic fungi. Drosomycin, which is the first inducible antifungal peptide isolated from insects has sequence and conformational similarities with plant defensins. (3) Thanatin, isolated from *Podisus maculiventris*, is particular by its activity spectrum which is directed against bacteria and fungi. Thanatin has one intramolecular disulfide bridge and sequence similarities with a class of frog skin antimicrobial peptides, the brevinin family. (4) In the scorpion, we have found a new cysteine-rich antimicrobial peptide, androctonin, which has no sequence similarities with other insect defense peptides but has a high degree of similarity with polyphemusins and tachyplesins, a family of antimicrobial peptides of *Limulus* (Arthropod, *Merostomae*). Androctonin has a broad activity spectrum. The data on the defense peptides in insects suggest that defensins have the largest distribution within this class. Similar substances were also found in scorpions and in molluscs. A phylogenetic tree for these molecules will be presented.

07-011

METCHNIKOWIN, A NOVEL ANTIMICROBIAL PROLINE-RICH PEPTIDE AND A NEW PATHWAY OF GENE EXPRESSION CONTROL ?

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Drosophila melanogaster has become a pivotal model system for the study of the insect host defense, because of the exceptional advantages which this species offers in terms of molecular and classical genetics. We report here the isolation and characterization of metchnikowin, a novel 26-residue inducible proline-rich antibacterial peptide, which also exhibits antifungal activity. The gene, which maps in position 52A1-2 on the right arm of the second chromosome, is expressed in the fat body after immune challenge, following classical acute-phase kinetics.

Metchnikowin is the second immune inducible peptide with antifungal activity to be characterized so far in *Drosophila*. It has recently been shown that distinct pathways control antibacterial and antifungal gene expression (Lemaitre et al., in preparation). Since metchnikowin exhibits both antibacterial and antifungal activities, we address the question, whether the pathway leading to the induced expression of metchnikowin, is a combination of both, or whether it is a different, third pathway. For this, the transcriptional profiles of the metchnikowin gene were analysed in the backgrounds of mutant fly lines, which have been shown to affect the expression of dipterocin (antibacterial) and/or drosomycin (antifungal) genes. Preliminary results indicate, that the expression pattern of the metchnikowin gene is different from those of dipterocin and drosomycin. The structural and functional analysis of the promoter region of the metchnikowin gene will be presented and a possible pattern of regulation of the gene expression will be discussed.

07-012

DIVERSITY OF INSECT PEPTIDE ANTIBIOTICS WITH REFERENCE TO *HETEROPTERA*

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About 100 peptide antibiotics found to date in insects demonstrate great structural diversity of effector molecules involved in insect innate immunity. Reporting here findings of antibacterial and antifungal peptides in *Palomena prasina* and *Podisus maculiventris* (Pentatomidae) in comparison with other insects allow to analyse peculiarities of humoral immune response in *Heteroptera* and some evolutionary trends in insect immunity.

All investigated bugs produce in response to septic injury a battery of antibacterial peptides selective to Gram-positive or Gram-negative bacteria with the exception of *P.maculiventris* thanatin killing various bacteria and fungi. Cytotoxic to Gram-positive bacteria materials are insect defensins, highly conservative 3 disulfide bonds containing molecules characteristic to many insects and to noninsect arthropods. The peptides cytotoxic to Gram-negative bacteria are more variable. Some of them such as glycine-rich hemiptericin of *Pyrrhocoris apterus* or one-disulfide bond containing thanatin have been isolated from one species only. Pyrrhocoricin of *P.apterus* and metalnikowins of *P.prasina* and *P.maculiventris* are structurally related proline and arginine rich peptides. In contradistinction to glycosylated uniform pyrrhocoricin, nonglycosylated metalnikowins are represented by 4 or 5 isoforms. Phylogenetic reconstruction shows that metalnikowins ancestor gene probably amplified 34-45 million years ago, much before *Palomena* and *Podisus* species diverged.

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07-014

THE DNA SEQUENCE ENCODING THE ANTIBACTERIAL PEPTIDE FROM *ARTOGEIA RAPAE*

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The partial DNA sequence, possibly encoding the cecropin-like antibacterial peptide, has been obtained from analysis of the DNA fragment being amplified by Reverse Transcriptase-PCR from the *Artogeia rapae* fat body challenged with *E. coli* K12. Three different kinds of the degenerate primers were designed on the basis of highly conserved DNA sequences of the known lepidopteran cecropin family: two of them are located on the preprosequence and the remaining is on the NH₂-terminal region of the structural gene.

The amino acid sequence deduced from the cDNA sequence shows 52-76% homologies to those of cecropins. Especially, highly conserved amino acid residues through the antibacterial peptides from moth are also well conserved in this sequence. It does not match exactly with any of the purified antibacterial peptides such as the hinnavin I and II, the antibacterial peptide purified from the *Artogeia rapae*, even if it shows higher amino acid homologies. The NH₂-terminal domain seems to be more conserved than the COOH-terminal domain which is believed to confer the spectrum of the antibacterial activities.

In order to obtain the complete cDNA sequence, the complementary analysis of cDNA library and marathon PCR technique is now being applied. Northern blot analysis will also show the expression patterns of this sequence in *Artogeia rapae*.

07-013

CAN LIFE-HISTORY DIFFERENCES EXPLAIN VARIATION IN ANTIBACTERIAL RESPONSES IN BEES (HYMENOPTERA: APOIDAE)?

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Honeybees live in warm and resource-rich environments which favour parasite invasion. British honeybees (*A. m. ligustica*) have larger feral colonies and lower nest absconding rates than Kenyan honeybees (*A. m. scutellata*). They also overwinter with large food stores, whereas Kenyan honeybees forage all year. British honeybees may therefore need more effective defense from parasites and pathogens. Comparisons of haemolymph antibacterial responses using agar plate growth inhibition assays supported this hypothesis. Naive antibacterial activity did not differ between honeybee races. However, after parenteral bacterial challenge, the antibacterial response of British honeybees was greater and of longer duration than those of Kenyan honeybees. Factors other than group size will also effect selection on immune systems; provisional data on the antibacterial responses of three less social British bees (*Bombus pascuorum*, *Lasioglossum* spp., *Psithyrus vestalis*) do not neatly fit into the sociality-immunocompetence theory. A modified hypothesis suggests that advanced hygienic behaviours found only in honeybees may partly negate the effects of increased group size.

The antibacterial peptides responsible for this activity were isolated in collaboration with Dr. P. Bulet, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France. There was microsequence variation in the peptides of Kenyan and British honeybees. *B. pascuorum* produced the same categories of peptide, but interspecific sequence variation was greater than intraspecific variation. C-terminal amidation of one category of peptide, a bee defensin, was shown for this bumblebee species.

07-015

MOLECULAR CHARACTERIZATION OF CERATOTOXINS, SEX-SPECIFIC ANTIBACTERIAL PEPTIDES FROM THE MEDFLY *CERATITIS CAPITATA* (DIPTERA:TEPHRITIDAE)

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The secretion of the female reproductive accessory glands of the medfly *Ceratitis capitata* has been shown to have an antibacterial activity. This property was ascribed to the presence of two cationic peptides (3kDa molecular mass) named ceratotoxins A and B, in addition to a lysozyme-like activity. The cDNA encoding ceratotoxin A has been isolated and the expression of this gene was analyzed. Ceratotoxin A is a sex-specific gene, related to sexual maturity in females, stimulated by mating and produced exclusively by the accessory glands. Since the juvenile hormone has a gonadotropic activity in the adult insect and morphological data show that the accessory gland development parallels that of the ovary, a possible interaction between the hormone and ceratotoxin expression has been hypothesized. This theory is supported by the effectiveness of the anti-juvenile hormone precocene II in suppressing ceratotoxin A gene expression. Another peptide having an antibacterial activity lower than that of ceratotoxin A has been purified from the gland secretion using a polyclonal antibody raised against ceratotoxin A, and its cDNA has been isolated. Based on the amino acid and nucleotide sequences it has been named ceratotoxin C.

Recently, two clones from a *Ceratitis capitata* genomic library hybridizing to a ceratotoxin cDNA probe have been isolated. The longer one, 26 kb, showed the presence of several ceratotoxin genes, revealing their clustered organization. The promoters of four ceratotoxin genes sequenced show some highly conserved motifs, suggesting the possibility of common regulatory elements for all ceratotoxin genes.

07-016

TRANSCRIPTIONAL REGULATION OF THE *DROSOPHILA* CECROPIN GENES

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We are interested in the function of the *Drosophila* immune response and as a model system we are studying the regulation of the *Cecropin* (*Cec*) genes. To identify *cis*-acting elements responsible for the inducible and tissue-specific transcription of the *Cec* genes we constructed a set of *Cec-lacZ* fusion genes. Their expression are analyzed by transfection and transient expression in cell lines and *in vivo* after P-element mediated transformation. Three regulatory elements, "R1", "κB" and "GATA", were identified as being necessary for normal *Cec* gene expression. Disruption of either of the κB or the GATA motifs results in inactivation of the promoter. The Rel protein Dif binds specifically to the κB site and acts as a strong transcriptional activator of *Cec-lacZ* constructs in cotransfection assays. Site-directed mutagenesis of Dif revealed that a conserved arginine at position 94 in the Rel domain is essential for Dif's activity. The Rel protein Dorsal can also activate the *CecA1* promoter, but to a lesser extent than Dif. Simultaneous overexpression of Dif and Dorsal in cotransfection assays revealed that Dorsal possesses a dominant negative effect on Dif *trans*-activation. We have also identified another DNA-binding activity recognizing the "GATA" motif, a well-known *cis*-acting element in vertebrates. The GATA site is located just adjacent to the κB site and some data indicate that the κB- and GATA-binding activities physically interact.

07-018

EARLY RESPONSE GENES IN THE *DROSOPHILA* IMMUNE DEFENSE; A NOVEL NF-κB-LIKE TRANSCRIPTION FACTOR

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In an effort to identify factors involved in the activation of immune responses, we use the PCR-based differential display technique to isolate genes which are induced by infection in *Drosophila*. Among induced genes, we find examples both of effector molecules, such as cecropin and attacin, and of regulatory molecules involved in the activation mechanism.

One induced gene, dubbed *Relish*, codes for a novel member of the Rel/NF-κB family of transcription factors, well-known activators of immune reactions in both mammals and insects. Similar to the mammalian NF-κB precursor p105, Relish includes a domain with six ankyrin repeats, related to the IκB class of NF-κB inhibitors. Transfection experiments show that Relish can activate transcription from a cecropin promoter, and this factor is likely to be an important regulator of the immune reaction. A specific *Relish* transcript is seen in early embryos, indicating that this gene, like *dorsal*, may also play a role in embryogenesis.

07-017

ADJACENT GATA AND κB-LIKE MOTIFS REGULATE THE INDUCTION OF IMMUNE GENES IN *DROSOPHILA*

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GATA motif is a well-known positive *cis*-regulatory element in vertebrates. Here we report the occurrence of such motifs in the promoters of a number of inducible insect immune genes and two mammalian acute phase response genes, IL6 and IL3. The GATA motifs in the proximal promoter region of these genes are located close to κB-like sites. The κB sites are positive *cis*-acting elements for the expression of *Cecropin A1* (*CecA1*). We present experimental evidence for the direct participation of the GATA motif in the expression of the *Drosophila CecA1* gene. A *Drosophila* blood cell line contains factors which bind specifically to the GATA motif of the *Cecropin A1* gene. These factors are likely to be members of the GATA family of transcriptional regulators. It is possible that, like in the case of insect immune genes, the GATA proteins may be involved in the mammalian IL6 and IL3 regulation as well. Thus, the previously proposed evolutionary relationship between the mammalian innate immunity and insect immunity via κB sites and Rel proteins may extend to include the participation of GATA elements as well. Antibodies against the *Drosophila* Rel protein, Dif, a known positive mediator of *CecA1* gene expression, recognises components of the GATA motif-specific complexes. This and the proximity of these two functionally important *cis*-acting elements in insects suggest an interaction between Dif and the GATA motif-specific factors in *Cec* gene regulation in *Drosophila*.

07-019

MULTIFACTORIAL CONTROL OF EXPRESSION OF ANTIBACTERIAL GENES IN *DROSOPHILA*.

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Insects respond to bacterial challenge by the rapid and transient synthesis of a battery of potent antimicrobial peptides. The analysis of the promoters of the genes encoding two of these molecules, diptericin and cecropin, revealed the presence of regulatory motifs homologous to *cis*-acting elements involved in the acute phase response in mammals (1). Among these motifs, decameric sequence elements similar to the binding sites for Nuclear Factor κB (NF-κB) have been shown to be mandatory for immune-induced transcription of both genes (2,3). However, the identity(ies) of the *trans*-acting protein(s) involved in this process remains unclear. We have investigated the transactivating capacities of the two *Drosophila* rel proteins dorsal and Dif (4,5) on reporter genes fused to either the diptericin or the cecropin κB-related motifs. We show by transfection experiments and DNA binding studies that these motifs are not functionally equivalent. Our results suggest that the diptericin and the cecropin genes are not regulated by the same activators or by the same combination of factors.

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07-020

JAK KINASE SIGNAL TRANSDUCTION PATHWAYS REGULATE *DROSOPHILA* HEMATOPOIESIS.

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In the fruit fly *Drosophila melanogaster*, blood cells are an important component of the host immune response. We are studying mutations in the *hopscotch* (*hop*) locus that disrupt hematopoiesis and cause leukemia-like defects, including the overproliferation and premature differentiation of hemocytes. The *hop* gene encodes a member of the Jak kinase family of non-receptor tyrosine kinases. Jak kinase proteins are known to play a vital role in the response of mammalian blood cells to cytokine stimulation.

We have used genetic and biochemical methods to identify other components of the Jak kinase regulatory pathways in *Drosophila*. We report on interactions of *hop* with other intracellular signaling molecules, including a fruit fly *raf* homolog. The regulatory role of these pathways in hematopoiesis and in the immune response will be described.

07-022

AN IMMUNE STIMULATED GENE EXPRESSED IN INSECT LARVAE ENCODES AN ALDOSE REDUCTASE-LIKE PROTEIN

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The immune state of insects is defined by a set of proteins that is absent in the naive state. To explore the immune system of *Trichoplusia ni* in more detail we have employed a PCR differential display technique to compare the mRNA population of untreated last instar larvae to that of immunized animals.

In the primary display, more than one hundred bands seemed induced upon bacterial challenge. When screening a cDNA library from immunized larvae with one of these amplified bands, we isolated a clone for an aldose reductase-like protein. The corresponding mRNA was found to be slightly up-regulated in developing last instar larvae. However, a bacterial infection strongly augments this response. This demonstrates the potential of the differential display PCR technique to find an immune regulated gene despite its background expression seen in larval development.

A comparison of the aldose reductase mRNA levels in different organs indicated that the fat body is the major place of synthesis.

The aldose reductase-like protein was over-produced using the baculovirus expression system. Two major bands of molecular weights 31 and 32 kDa were found. The product had aldose reductase enzyme activity and was affinity purified on Blue Sepharose.

The major function of aldose reductase in other systems is to produce osmo-protectants such as sorbitol or mannitol in response to stress situations. However, the concentrations of such sugar alcohols did not increase in response to immune stimulation. Work is now in progress to understand the function of the *T. ni* aldose reductase in insect immune induced stress.

07-021

IMMUNE SYSTEM GENES AND MUTATIONS CAUSING TUMOR FORMATION IN *DROSOPHILA*

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We are using *Drosophila* as a model system for the analysis of immunity and tumor formation and have conducted two types of screens using enhancer detector strains to find genes related to these processes: (A) genes expressed in immune system tissues, i.e. hemocytes, lymph glands (the hematopoietic organ) and fat body (major site of antibacterial protein synthesis); (B) genes increased in expression by bacterial infection. Among the genes of types A and B, we hypothesized that a third type would be found: genes that when mutated have a melanotic tumor phenotype. 2,800 enhancer detector strains were screened for type A and 900 for type B, and 11 strains retained for further analysis. Complementation tests, cytological mapping of *P* elements, mobilization of *P* elements, and determination of time of lethality and mutant phenotype have identified six novel genes, *Dorothy*, *wizard*, *toto*, *viking*, *Thor* and *dappled*, and one previously identified gene, *Collagen IV*. All of the novel genes are associated with reporter gene expression in at least one immune system tissue. One is also associated with an increased level of expression upon bacterial infection. Mutations of two of the novel genes have a melanotic tumor phenotype. We are currently working on the molecular characterization of three of these genes, in particular *dappled* will be presented. Mutations of *dappled* show tumor formation associated with aberrant gut and fat body; one mutation is viable with 100% tumor formation and another is lethal with tumor formation in early larval development. The enhancer detector insertions in these strains are approximately 100 base pairs apart. Molecular characterization will help determine the recognition and response by the immune system when aberrant self tissue is present.

07-023

ANALYSIS OF ENHANCER TRAP LINES FOR THE STUDY OF *DROSOPHILA* HEMOCYTES.

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The immune response of insects involves two distinct mechanisms: a humoral facet characterized by the synthesis in the fat body of various antimicrobial peptides, and a cellular response by the hemocytes, which is still poorly understood. Insect hemocytes have been investigated for some fifty years. The classifications that have been published to date are based on morphological and more rarely functional criteria. In *Drosophila*, several blood cell types have been described. In third instar larvae, the lymph glands constitute the major (perhaps unique) source of circulating hemocytes which can be classified into three cell types : (i) the crystal cells (5 - 10 % of the hemocytes) contain the components of the circulating prophenoloxidase cascade which participates in the defense-related melanisation processes ; (ii) the plasmatocytes which are small round cells that account for most of the blood cell population and are believed to contribute to the host defense of *Drosophila* (phagocytosis of bacteria, production of antimicrobial peptides) and (iii) lamellocytes which are characteristically flattened cells. In the larvae lamellocytes serve in the process of encapsulation. At the end of the larval instar the plasmatocytes differentiate into lamellocytes which predominate in the blood cell population of prepupae. This differentiation is under hormonal control.

We have screened ca. 1300 β -galactosidase enhancer trap lines for expression of the transgene in various subsets of larval hemocytes. This expression has been analysed in different mutant contexts known to affect the larval hemocytes. We describe a P-element mediated mutation on the second chromosome which results in the absence of circulating hemocytes in larvae, and in early pupal lethality. The incidence of this mutation on the immune response is analysed.

07-024

MOLECULAR APPROACHES TO ANOPHELES GAMBIAE IMMUNITY

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Vector-mediated transmission of *Plasmodium* (the agent of malaria) requires successful growth and differentiation of the parasite within the physiological environment of a mosquito host. Various barriers to *Plasmodium* development have been documented in particular mosquito species and strains, which suggest that in some cases the immune system of the mosquito can limit vectorial capacity. Great progress has been made in understanding insect immune mechanisms, largely through biochemical and molecular studies focused upon a few model species. In the case of *Anopheles gambiae*, the principal vector of human malaria in Africa, molecular aspects of immunity are only now in the process of being elucidated. In initial experiments, *An. gambiae* homologues of genes implicated in mediating the insect immune response to microbial agents have been identified. The gene products are being analyzed for their utility as probes to monitor the mosquito response to infection by *Plasmodium* parasites.

We have cloned a gene encoding the *An. gambiae* homologue of insect defensin, an inducible antibacterial protein. Defensin RNA levels are elevated rapidly following bacterial infection challenge of larval and adult stage mosquitoes. A member of the rel-family of transcription factors has also been identified in *An. gambiae*. Rel-family members are implicated in the regulation of infection-inducible expression of a number of insect immune factor genes. The activities of defensin and the rel-factor are being analyzed during the course of mosquito infection by *Plasmodium* parasites.

Biochemical fractionation of extracts prepared from bacterially-challenged mosquitoes reveals numerous distinct, inducible antibacterial activities. The antibacterial proteins are being purified and sequenced, leading to the identification of the corresponding cDNAs (thus providing additional markers which may prove useful in analyzing the mosquito response to parasite infection).

To specifically address the mosquito immune response to *Plasmodium* infection challenge, the mRNA differential display technique is being used to identify *An. gambiae* genes that are transcriptionally activated as a consequence of ingestion of an infective blood meal. Results of these experiments will be discussed.

07-026

ANOPHELES STEPHENSI : INDUCIBLE ANTIBACTERIAL PEPTIDES AND GENETIC SELECTION ACCORDING TO ANTIBACTERIAL ACTIVITY.

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Molecules are known to be induced in the haemolymph of insects by infection or injury and some of these have antiparasitic and bactericidal activity. In mosquitoes, although the outward manifestation of refractoriness to infection is melanization, the actual mediator of parasite death may involve these immune peptide(s). Some have been found in *Aedes aegypti*, and other vectors of parasitic infection.

The results of antibacterial zone assays show that the haemolymph of immunised *An. stephensi* contain substances with activity against gram-negative and gram-positive bacteria. Genetic selection based on the levels of anti gram-negative activity have resulted in the production of two mosquito lines; one that demonstrates low responding activity and one that demonstrated high responding activity against *E.coli* D31. Results from this selection experiment and the complete amino acid sequences of antibacterial peptide(s) from the selected lines of these mosquitoes are presented.

07-025

THE IMMUNE FACTOR GAMBIF1 IS A NOVEL REL-FAMILY MEMBER FROM THE HUMAN MALARIA VECTOR AN. GAMBIAE,

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A novel rel-family member, Gambif1 (gambiae immune factor), from the human malaria vector *An. gambiae* has been cloned. It has the highest homology to *Drosophila* dorsal and Dif. Gambif1 mRNA is present at low levels in all developmental stages. Its protein is translocated to the nuclei of fat body cells in response to bacterial challenge. DNA binding activity to the *Drosophila* dipterin and cecropin, and the *An. gambiae* defensin kB-like sites is also induced in larval nuclear extracts following bacterial infection. Gambif1 has the ability to bind to kB-like sites. Co-transfection assays in mbn-2 cells show that Gambif can activate transcription by interacting with the *Drosophila* dipterin regulatory elements and that it is not functionally equivalent to dorsal. These molecular markers of activation of the immune system open the possibility to study the role of the mosquito defense reactions in determining their susceptibility/refractoriness to malaria infection.

07-027

IMMUNE PEPTIDES FROM AEDES AEGYPTI AND ANOPHELES STEPHENSI.

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Injection of non-pathogenic bacteria is known to induce an immune response in several insect orders. Using HPLC coupled to antimicrobial bioassays, we have characterised this response in 2 medically important Diptera. In the mosquitoes *Aedes aegypti* and *Anopheles stephensi*, immune haemolymph is active against both Gram positive and Gram negative microorganisms. *Ae. aegypti* possesses two insect defensin isoforms and an inducible haemolymph lysozyme which are active against Gram positives. In this species 2 types of anti-Gram negative activity are now described. The first consists of a family of at least 5 small peptides for which preliminary sequence data is presented. The remaining activity appears to be due to a single larger peptide, with different HPLC characteristics. The malaria vector *An. stephensi* possesses at least one defensin with 90% identity to the *Ae. aegypti* defensins, and at least one type of anti-Gram negative activity is also present in this species.

07-028

IMMUNE ACTIVATION OF *AEDES AEGYPTI* REDUCES THE ESTABLISHMENT AND DEVELOPMENT OF *BRUGIA MALAYI*.

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Mosquitoes respond to bacterial challenge or cuticular injury by the rapid production of potent immune proteins. We have identified three insect defensins produced by the mosquito *Aedes aegypti* in response to bacterial challenge. We evaluated the effect of immune activation on the establishment and development of *Brugia malayi* ingested in a blood meal both in susceptible and refractory strains of *A. aegypti*. Immune activated mosquitoes had significantly reduced prevalences and mean intensities of infection than did naive controls when exposed to bloodmeals with low (105 mf/20µl blood) and medium (160 mf/20µl blood) microfilaremias. Northern analysis showed that all mosquitoes demonstrated rapid defensin transcriptional activity after immune activation but no strain of *A. aegypti* produced defensin transcripts after bloodfeeding on control or parasite-infected gerbils. The data suggest that inducible immune proteins of mosquitoes can reduce the prevalence and mean intensity of infection with ingested parasites, but these proteins are not expressed routinely after parasite ingestion and midgut penetration. Immune proteins such as defensins, however, represent potential candidates to genetically engineer mosquitoes resistant to filarial worms.

07-030

A MONOCLONAL ANTIBODY AGAINST A HEMOCYTE 180 kDa POLYPEPTIDE INVOLVED IN CELLULAR DEFENCE REACTIONS IN THE STICK INSECT *BACILLUS ROSSIUS* (Rossi) (Phasmatodea, Bacillidae).

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The monoclonal antibody BrH1 has been obtained immunizing mice with paraformaldehyde-fixed *Bacillus rossi* hemocytes and screening hybridomas by immunofluorescence with hemocyte monolayers. The antibody stains mainly granule-containing hemocytes in *in vitro* cultured cells, and cells having an irregular morphology in *in situ* transversal cryosections of adult insects.

BrH1 recognizes in western blotting analysis a 180 kDa antigen present in hemocyte lysates but absent from fat body lysates and from cell-free haemolymph. This antigen appears to be involved in cellular defence reactions against experimental *in vivo* challenge with killed yeast cells, since in immunofluorescence analysis of cryosections, a strong positivity has been observed around and between yeast cells after 18 hours of incubation.

The antigen recognised by BrH1 appears very likely contained in secretion granules released *in vivo* by stick insect hemocytes during defence reactions against microbial infection.

07-029

DEMONSTRATION OF AN INDUCIBLE DEFENSIN LIKE IMMUNE PEPTIDE IN THE HAEMOLYMPH OF *SIMULIUM DAMNOSUM* S.L. (DIPTERA:SIMULIIDAE) S.L. Kläger, H.E. Hagen, D.V. Barrault, D. Hultmark¹, P.J. Ham

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Infections of *S.damnus* s.l. with bacteria or *Onchocerca* microfilariae lead to the induction of two peptides, visible on SDS-Tricine- polyacrylamide gels as a doublet of around 4.5 - 5 kDa. Growth inhibition zone assays and modified overlay assays revealed that at least one of these two peptides has a strong anti-gram positive activity. Due to the poor resolution of the overlay gels, the activity could not be allocated to one peptide of the doublet. As in other insects peptides of that size with antigram positive activity have been shown to belong to the group of insect defensins, we used defensin homology based generic primers in a Differential Display Reverse Transcription PCR. Several products are in the process of being sequenced, sharing partial sequence homology with other insect defensins.

07-031

APPLICATION OF MONOCLONAL ANTIBODIES TO STUDY INSECT HEMOCYTE FUNCTIONS

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Insects respond to infections and parasitization with cellular and humoral defence mechanisms. Hemocytes respond to pathogens through phagocytosis, nodule formation, and encapsulation. They clearly distinguish self from non-self, thus play an important role in triggering immune responses in insects.

Several hemocyte types can be identified by light and electron microscopy. However, it is often difficult to identify hemocyte types with certainty because their morphology can vary depending on the experimental situation. This is especially true for *in vitro* experiments in which hemocytes have not been activated or did not have time to adhere and spread, therefore they can not be distinguished. In order to overcome these problems several monoclonal antibodies (mabs) have been developed against hemocytes of a few species.

This presentation will give an overview of studies in which mabs against hemocytes of *H. cecropia* and *M. sexta* have been used to identify distinct hemocyte types. Furthermore, it will be shown how mabs can be used to identify hemocyte changes during the development of *H. cecropia* that can not be detected morphologically. Also first results of experiments in which mabs have been used to interfere with hemocyte behaviour and functions (e.g. encapsulation) will be presented.

This work was partially done in collaboration with M. Kanost (Kansas State University, USA), C. Wiegand (Free University Berlin, BRD), and E. Willott (University of Arizona, USA).

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07-032

MOSQUITO CELL LINES BIND ANTIBODIES MADE AGAINST *MANDUCA SEXTA* HEMOCYTES

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In vitro work on insect hemocytes is feasible for large insects, such as the caterpillar *Manduca sexta*. Studying smaller insects, such as the mosquito, poses more problems. For some purposes, the cell lines established from these insects may be useful. But how hemocyte-like are the cell lines? Some monoclonal antibodies made against *M. sexta* hemocytes crossreact with mosquito, *Aedes aegypti* and *Anopheles gambiae*, hemocytes. They also bind to cells of several different mosquito cell lines.

07-033

IDENTIFICATION OF A HEMOCYTE MEMBRANE PROTEIN OF THE SILKWORM, *BOMBYX MORI*, WHICH SPECIFICALLY BINDS TO BACTERIAL LIPOPOLYSACCHARIDE

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An *in vitro* system with isolated hemocytes of the silkworm, *Bombyx mori*, was developed to examine the induction mechanism of insect antibacterial proteins by bacterial lipopolysaccharide. The gene expression of *B. mori* cecropin B was triggered by LPS in this system. To identify LPS-binding site(s) of the hemocytes, the [¹²⁵I]LPS binding assay to the hemocytes was performed *in vitro*. The amount of [¹²⁵I]LPS bound to hemocytes increased proportionately with the increase of incubation time and LPS dose. The binding was strongly inhibited by excess unlabeled LPS or lipid A, indicating that the binding of [¹²⁵I]LPS to hemocytes contains a highly specific reaction. Moreover, the specific binding could not be detected with Bm-N4 cells in which cecropin B gene expression was not induced by LPS, suggesting that the LPS binding is specific for LPS responsive cells. The LPS binding was fully sensitive to the proteinase K treatment of intact hemocytes, suggesting that a protein(s) located on the surface of hemocytes is involved in the LPS binding. Fluorescein isothiocyanate conjugated-LPS binding assay demonstrated that this compound mainly binds to granular cells rather than other hemocytes under our assay conditions. Affinity-labeling with photoreactive-LPS allowed the identification of a 11 kDa LPS-binding protein in hemocytes, which might relate to the specific membrane receptor for LPS.

07-034

HEMOCYTE-MEDIATED CLEARANCE OF LIPOPOLYSACCHARIDE IN INSECTS: PROTEIN TYROSINE PHOSPHORYLATION REQUIREMENT

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The response of *Ceratitis capitata* hemocytes to lipopolysaccharide (LPS) involves the induction of intracellular protein tyrosine phosphorylation and, hence, the release of biologically active molecules, including protein p47 and phenoloxidase activators. The effector protein p47, one of the two dominant hemocyte proteins of developing medfly, could not be normally traced in plasma. However, it is present on the cell surface, recognises and attaches to *E. coli* cells and it binds covalently tyrosine derivatives generated by the action of phenoloxidase.

The effector protein p47, activated phenoloxidase (either cell-associated or cell-free) and tyrosine, constitute a system responsible for the clearance of invaders from the hemocoel of medfly. According to our data, LPS (either cell-associated or cell-free) recognises and crosslinks membrane bound p47 via the intermediacy of tyrosine derivatives of eumelanin metabolic pathways generated by the action of phenoloxidase, as is the case for cuticular protein-chitin crosslinks during sclerotization.

This covalent association of LPS at the cell surface, immobilizes bacteria and evidently this is an initial step for their internalisation as judged by the resistance of LPS binding to dissociation by proteinase K. The novel aspect of our results appears to be the intracellular signaling pathways required for LPS internalisation.

07-035

INFECTION OF LEPIDOPTERAN LARVAE WITH A SMALL RNA VIRUS INDUCES MASSIVE SHEDDING OF MIDGUT CELLS.

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We are exploring how the midgut of lepidopteran larvae responds to infection by small RNA viruses. The differentiated columnar and goblet cells forming the single layer midgut tube are produced by division of regenerative cells located in the surrounding basal membrane. During larval growth, the columnar and goblet cells increase significantly both in size and in number. *Helicoverpa armigera* stunt tetravirus (HaSV) is highly specific for the midgut and is able to infect all three cell types. The virus displays pathogenicity only to the first three larval instars; in contrast, fourth instar larvae appear to recover from infection with proportionally higher doses. This is an ideal system to study the insect cellular response to virus infection *in vivo*, as no humoral response is likely.

Immunohistochemical studies at the electron, light and confocal microscope levels on midgut pathology in infected first instar heliothis larvae, along with molecular techniques, show that HaSV rapidly induces a massive increase in shedding of rejected midgut cells above the level of sloughing normally found in healthy larvae. This increased shedding exceeds the ability of regenerative cells to maintain a functional midgut, resulting in irreversible degeneration of the midgut. We are currently investigating the roles of cell-shedding and other factors such as gut cell number and structure in determining the sensitivity of neonates and the resistance of larger larvae to HaSV infection. Further work asks whether HaSV-induced apoptosis underlies cell-shedding.

07-036

BEHAVIOUR AND CYTOSKELETON ALTERATIONS OF PLASMATOCYTES FROM *GALLERIA MELLONELLA* CAUSED BY THE ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE* AND ITS TOXINS

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The entomogenous fungus *Metarhizium anisopliae* is known to release destruxins and cytochalasin. These secondary metabolites have been examined for their potential as pest control agents due to their toxic effects on insects. Entomogenous fungi may produce these toxins to kill the host or to incapacitate its immune system. With the aim to evaluate their function during mycosis we studied their influence on isolated plasmatocytes, which represent the main phagocytic cell type and participate in the encapsulation process within the hemolymph of *Galleria mellonella*. Attachment, filopodia formation, phagocytic activity and cytoskeleton formation of isolated plasmatocytes was examined under presence of destruxin or cytochalasin and in comparison with untreated plasmatocytes and plasmatocytes obtained from *Metarhizium anisopliae* infected larvae. Dissolved destruxin or cytochalasin in sublethal concentrations caused characteristic morphological and cytoskeleton alterations and suppressed attachment, filopodia formation and phagocytic activity of plasmatocytes in monolayers. Plasmatocytes isolated from diseased larvae exhibited similar effects concerning attachment, spreading and cytoskeleton formation. The corresponding effects on cell mobility and cytoskeleton formation in plasmatocytes *in vitro* and in plasmatocytes from infected larvae support the conclusion that entomopathogenic fungi release these toxins to interfere with cellular immune reactions of their hosts.

07-038

NEWLY DETECTED IMMUNE STIMULATING MOLECULES OF *GALLERIA MELLONELLA* (LEPIDOPTERA).

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In vivo and *in vitro* transfer studies were conducted to detect immune stimulating molecules in cell free larval haemolymph and in cell culture supernatants of phagocytically active haemocytes. Molecules derived from these sources were assayed for their ability to activate the antibacterial humoral response *in vivo* as well as the phagocytic activity of isolated haemocytes *in vitro*. Two activating factors could be characterized in more detail: Apolipophorin III (ApoLp-III) from larval haemolymph and a prostaglandin-like molecule released by phagocytosing haemocytes *in vitro*. ApoLp-III was revealed to be a heat stable protein with a molecular mass of 18077 Da. The UV-spectrum and the partially analyzed amino acid sequence data indicate that apoLp-III from *Galleria mellonella* and *Manduca sexta* are closely related. The prostaglandin-like factor is a small (< 500 Da), heat sensitive and hydrophobic molecule. The isolation procedures for both molecules, detailed data about their biological activities, and first results about their putative mode of action will be presented. The unexpected role of apolipophorin III as an immune activator and the central role of haemocytes in delivering immune regulating molecules will be discussed.

07-037

IDENTIFICATION OF A HEMOCYTE PROTEIN INVOLVED IN ENCAPSULATION OF FOREIGN MATERIAL IN THE TOBACCO HORNWORM, *Manduca sexta*.
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The biochemical events leading up to and during hemocytic encapsulation are poorly understood. We have initiated an investigation into the molecular basis of encapsulation and cell adhesion. Previously, we had prepared a panel of monoclonal antibodies that recognize hemocytes from *M. sexta* larvae. These antibodies have a wide range of hemocyte labeling characteristics. We have found one antibody, MS13, a plasmatocyte-specific membrane marker, interfered with the ability of the plasmatocytes to spread on glass slides. Also, pre-incubation of hemocytes with MS13 decreased the amount of latex beads encapsulated *in vitro*. We present information regarding the target protein for MS13 (termed HC13). This protein has been identified as an integral membrane protein with a molecular weight of 90-100 kDa. We also present data to show which types of cell are involved in the encapsulation process. This information is discussed in context with published results.

07-039

THE ROLE OF BDL1, AN ENDOGENOUS MANNOSE-SPECIFIC LECTIN, ON THE PHAGOCYTOSIS OF FOREIGN PARTICLES BY HAEMOCYTES OF THE COCKROACH, *BLABERUS DISCOIDALIS*.

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BDL1, a mannose specific lectin isolated from the serum of *Blaberus discoidalis*, has previously been shown to activate the prophenoloxidase system. In this present study, the phagocytosis of foreign particles was examined, with a view to determining if an enhanced phagocytic rate in the presence of BDL1 was due to BDL1 activating the prophenoloxidase cascade or acting independently as an opsonin. In the presence of BDL1, the rate of phagocytosis, of some microorganisms is significantly increased above levels observed when prophenoloxidase is activated by laminarin alone. The role of BDL1 in this activation was determined using specific monoclonal antibodies to inhibit BDL1, and serine protease inhibitors to prevent the activation of the prophenoloxidase cascade.

07-040

SELECTION OF *Aedes aegypti* LINES HAVING QUANTITATIVE DIFFERENCES IN IMMUNE PEPTIDE ACTIVITY, AND THEIR ASSOCIATION WITH FILARIAL SUSCEPTIBILITY

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Data will be presented describing the phenotypic selection of two segregated lines of *Aedes aegypti* using the HUMORAL antibacterial immune response to bacteria inoculation as the selection criteria. The two lines, currently at the F¹⁰ generation, showed early differences through maternal selection. Responses against both *E. coli* and *M. luteus* were measured and other characteristics such as responses in males and the resulting susceptibility levels of females to *Brugia* infection have been determined. The latter show a clear association with the phenotypes used to generate these novel lines indicating a causal relationship between antibiotic activity and parasite development.

07-041

THE EFFECT OF HEMOLYMPH PLASMA ON THE HEMOCYTE BEHAVIOUR AND ITS POSSIBLE ROLE IN THE INITIAL IMMUNE RESPONSE OF INSECTS.

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The activation of immune response still has a number of unresolved problems in arthropods. The sequence of biochemical events operative after microbial invasion or wounding and the subsequent immune reaction is not fully understood. It is likely that humoral factors influence hemocyte behaviour. We have found that plasma induced aggregation, spreading, and the formation of cellular networks in hemocytes from *Manduca sexta* and *Pieris brassicae* *in vitro*. Plasma from both species was ultrafractionated and the different fractions were tested. In *P. brassicae* the aggregation activity was observed in the fraction of 30-100 kDa and the spreading and the formation of cellular network was found in the fraction of 3-10 kDa. These factors were deactivated by heating (100 °C, 10 min) and by proteolytic enzymes. The spreading and network activity (3-10 kDa) was also deactivated after lipase treatment, suggesting that these factors are of proteic or lipoproteic nature. For *M. sexta* we found all activities expressed in a plasma fraction of less than 3 kDa. The activating factor (AF) resisted denaturing treatments and proteolytic enzymes. Hemocytes entrapped rat erythrocytes (RE) in large nodules in presence of the AF from *M. sexta* or the 3-10 kDa fraction from *P. brassicae*. These results suggest that caterpillar hemolymph contains necessary factors responsible of the hemocyte behaviour and activation *in vitro*. Current research in our laboratory is focused on the purification of these factors and their molecular characterisation.

07-042

ANTIPARASITIC AND ANTIMICROBIAL ACTIVITY IN INSECTS OF MEDICAL IMPORTANCE

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Insects produce antibacterial factors in response to bacterial infection. Study of the humoral immune responses of *Simulium equinum* and *Aedes aegypti* has shown that both species synthesize anti-bacterial and anti-parasitic substances. Apidaecins, lysozyme and defensins have been isolated from *Aedes aegypti*. Lysozyme and a defensin-like peptide have been isolated from *Simulium equinum*. Results of the isolation of anti-bacterial factors and of the *in vitro* activity of *Aedes aegypti* defensin against eukaryotic and prokaryotic organisms will be presented.

07-043

PEPTIDOGLYCAN AS A SIGNAL MOLECULE FOR INDUCTION OF ANTIBACTERIAL PROTEINS IN THE SILKWORM, *BOMBYX MORI*

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Bacterial cell wall components such as peptidoglycan (PG) and lipopolysaccharide (LPS) are known to be effective elicitors for the induction of antibacterial proteins in insects. We have utilized the silkworm as a model for characterizing the induction mechanisms of antibacterial proteins. Currently, we have shown that the silkworm recognizes PG fragments of some definite structure as a signal molecule and transduces the signal to the genome, which then introducing the simultaneous synthesis of a series of antibacterial proteins such as cecropin and lysozyme in fat body. The minimum structure of PG required for the induction of antibacterial proteins was elucidated by injecting the silkworm larvae with PG fragments having structurally different peptides or different sugar chain length with or without cross-linking prepared from various species of Gram-positive bacteria. The PG fragments would be recognized by putative PG-binding protein on the fat body cells. We are also characterizing the nature of signal transduction after the recognition of PG by testing the effects of various inhibitors on the induction of cecropin and lysozyme mRNAs in cultured fat body.

07-045

ASABF, a cysteine-rich antibacterial protein isolated from the nematode *Ascaris suum*: A comparison with insect/arthropod defensins.

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Previously, we reported that at least three humoral defense activities (antibacterial, bacteriolytic, and agglutinating) were detected in the body fluid of the parasitic nematode *Ascaris suum*. The substantial nature of these activities was suggested to be proteins/peptides, because all of them were lost by trypsin digestion. One of them, ASABF (*A**s**c**a**r**i**s* *s**u**m* *a**n**t**i**b**a**c**t**e**r**i**a**l* *f**a**c**t**o**r*) is a heat-stable antibacterial protein with molecular mass of 7 kDa. Recently, we purified ASABF, and its amino acid sequence was determined. Eight Cys residues which might contribute to intramolecular disulfide bonds were found in this sequence. MPsrch data base searches revealed weak sequence identity between ASABF and insect/arthropod defensins (IADs), the antibacterial proteins which contain 6 Cys residues. Jumbling test supported that this similarity was significant. All of them were positively charged proteins. In addition, Gram-positive bacteria were much more sensitive to ASABF (IC₅₀=0.6 µg/ml, against *Staphylococcus aureus*) than Gram-negative bacteria (IC₅₀=50 µg/ml, against *Escherichia coli*), and this antibacterial spectrum is similar to IADs. However, ASABF consisted of over 60 residues, whereas IADs consisted of only 30-40 residues. The positions of Cys residues in the sequence of IADs were highly conserved, and they are essential to the antibacterial activity. In contrast, an additional Cys of ASABF was found at the internal position of conserved array of Cys residues. ASABF, therefore, is not a typical IAD, although ASABF might be evolutionally related to IADs.

07-044

PARASITISM BY *COTESIA GLOMERATA* (L.) INDUCES CHANGES IN HEMOLYMPH PROTEINS OF THE HOST *PIERIS BRASSICAE* (L.)

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The cabbage moth *Pieris brassicae* is naturally parasitized by the braconid wasp *Cotesia glomerata*. Parasitism by *Cotesia glomerata* induces multiple changes in the physiology of *Pieris brassicae*. These changes mainly affect the development and the endogenous defence system of the host insect.

After parasitization changes in the hemolymph proteins of the host were found. Analysis of the hemolymph proteins with gelelectrophoresis showed "parasitism specific" polypeptides. One of these proteins appeared in the hemolymph two days post-parasitization while the other two proteins appeared a few days before the parasitoid larva emerged. The relative molecular weights of these parasitization induced proteins are around 38'000, 42'000 and 128'000 respectively. In several parasitoid-host systems "parasitism-induced" proteins have been identified. These become detectable in the host at varying times after parasitization. Whether these proteins play a role in the suppression of the host immune system or in the regulation of the host physiology is in most cases still unknown.

07-046

SCREENING FOR IMMUNE RELATED GENES BY DIFFERENTIAL DISPLAY

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We are performing a thorough analysis of the insect immune machinery, using *Drosophila melanogaster* as a model system. This is done by a technique of differential screening, the differential display reverse transcription PCR (DDRT-PCR), comparing normal flies to flies induced with bacteria. So far we have confirmed differential expression for eleven PCR-fragments isolated in an initial screen. Two of the fragments turned out to encode immune-related proteins of already known function. We are isolating cDNA clones for the rest of the fragments. One of the isolated clones encodes a protein consisting of 126 aa, but sequence analysis suggests that it might be further processed into a smaller peptide. The rate of induction, amino acid composition, localization and expression pattern of this protein show several unique and very interesting features. The immune function of this protein are presently under investigation. Two different sets of genes are expected to be activated and therefore found in this screen. Genes encoding effector molecules and genes that code for molecules involved in the induction process such as receptors, messenger molecules, transcription factors etc. Several of the fragments isolated so far show a very interesting and promising pattern on northern blots.

07-047

ANTIBACTERIAL PEPTIDES FROM *SPODOPTERA LITURA*Sung Moon Yoe, Chung Sik Choi¹, Hak Ryul Kim¹

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Sixth instar larvae of *Spodoptera litura* were found to contain inducible antibacterial activity in their haemolymph. This immune reaction was induced by primary infection with viable 10^6 log phase of *E. coli* K-12 into the thorax. These proteins were separated and two of them were purified to homogeneity by ion-exchange chromatography, gel filtration and reverse phase FPLC. The purified proteins have strong antibacterial activity against gram negative and gram positive bacteria, and these proteins were named as spodopsin Ia and Ib, respectively. The sequences of spodopsins are confirmed by three methods, such as amino acid composition analysis, Edman degradation and electrospray mass spectrometry. Both spodopsins consist of 35 amino acid residues and molecular weights are approximately 4 kDa. We will discuss these peptides.

07-049

SECRETION OF SANDFLY MIDGUT LECTIN IS STIMULATED BY PROTEINS IN THE MEAL

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Midgut lectin activities (LA) of *Phlebotomus duboscqi* and *Lutzomyia longipalpis* (Diptera: Psychodidae) were elevated after bloodfeeding to reach peak levels two or three days later. In unfed flies the lectin was associated mainly with microvillar surface, in fed ones it was secreted into midgut content. In both unfed and bloodfed flies the sugar binding specificities of LA were similar and anti-lectin antibodies recognized the same protein band in immunoblotting.

Different protein solutions were fed to female flies through membranes. Positive correlation was found between the extend of lectin secretion and the protein concentration in the meal. Stimulatory effect on gut lectin secretion had all protein solutions used. Bovine serum albumin and hemoglobin were equally effective as blood derivatives of the same protein content. Possibility that sandfly midgut LA and proteases might form a functional complex which participates on bloodmeal digestion and interaction with transmitted pathogens is discussed.

07-048

DIFFERENTIATION OF HEMOCYTES IN HEMOPOIETIC ORGAN OF *BOMBYX MORI* L.

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Bombyx mori L. larva was investigated with a transmission electron microscopy to determine hemocytic differentiation in the hemopoietic organ located in thorax. Three or four types of stem cells in compact islets of the organ were observed. Immature hemocytes in loose islets of the organ were more differentiated and developed than in compact islets. Prohemocyte, plasmatocyte, granulocyte and oenocytoid were observed in loose islets. Each type of hemocyte was differentiated from each type of stem cell. However, none of spherulocyte was observed. Each type of hemocytes matured in loose islets were discharged into hemolymph by the tearing of acellular membrane covering the islets. The more detailed pathway of differentiation of hemocytes in *B. mori* was represented by the this investigation.

07-050

POSSIBLE ROLE OF A PURIFIED LECTIN FROM HAEMOLYMPH OF THE DESERT LOCUST SCHISTOCERCA GREGARIA MECHANISMS AGAINST THE PROTOZON MALAMOEBA LOCUSTA.

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Haemolymph from the desert locust showed agglutination activity for mammalian erythrocytes and protozoa. A purified lectin was isolated from plasma by affinity chromatography. Analysis of the purified lectin by SDS-PAGE gave a single band at 80 KD while on native PAGE, a single band at 650 KD. Galactosides with an & D configuration were found to inhibit agglutination activity. This lectin enhanced *Malamoeba locustae* cysts fixation on locust hemocytes. Immunoblot analysis using antiserum raised against the purified lectin indicated that lectins from *S. gregaria* and *Locusta migratoria* share common immunological characteristics. Elisa tests used to assess the level of lectin gave higher titres in infected locusts as compared to controls. Phagocytosis appeared to be a major factor in the clearance of *M. locustae* from locust haemolymph. The highest level was reached 6 hours post inoculation and clearance of cysts from live insects was completed after 48 hours. The role of lectin in phagocytosis process and locust defense mechanisms, and the use of lectin inhibitors in locust control management are discussed.

Key words : Desert locust, *Schistocerca gregaria*, *Malamoeba locustae*, lectin, purification, immunoblot, Elisa, agglutination, phagocytosis.

07-051

TRANSFERRING ANTIBACTERIAL PEPTIDE GENE FROM CHINESE OAK SILKWORM INTO YEAST AND IDENTIFICATION OF ITS EXPRESSED PRODUCTS

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A synthetic antibacterial peptide gene (APG) from Chinese oak silk worm, *Antheraea pernyi* was recombined with a shuttle plasmid pCLWA2 containing an alpha-factor promotor and transferred into yeast AB103. The cells of yeast grew well under 28-30°C, pH 5.0 in YEPD medium. The expressed products were identified by electrophoresis with detection of antibacterial activity. The expressed peptide has a molecular weight of 4000 daltons. The yield of products reached a level of 1.14mg/L YEPD and its activity unit is about 0.216U.

07-053

PURIFICATION OF A HUMAN ALTERNATE COMPLEMENT PATHWAY INHIBITING PROTEIN FROM THE HEMOLYMPH OF THE SILKWORM LARVAE, *Bombyx mori* Y.Sekijima, Y.Fujikura, T.Hiyama¹, T.Maenaka¹ and A.Uda¹

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The hemolymph of the silkworm larvae has been shown to possess factor capable of inhibiting human alternate complement pathway. To elucidate the property, a human alternate complement pathway inhibiting protein(HALP-SHIS)was purified from the hemolymph of the silkworm larvae.The activity of HALP-SHIS was determined by ACH50 to rabbit erythrocytes and a hemolytic agar plate assay, which has been developed by us. The supernatant of 30%(NH₄)₂SO₄ saturation was applied on a DEAE-Toyopearl 650M (TOSOH) column equilibrated with 100 mM phosphate buffer(pH 7.2). Bounded HALP-SHIS was eluted in the range of 200-250 mM NaCl. The active fractions of HALP-SHIS were pooled and then applied on a Butyl-Toyopearl 650C,hydrophobic adsorbent(TOSOH) column equilibrated with 50 mM phosphate buffer(pH 7.2) and 750 mM ammonium sulfate. HALP-SHIS was eluted in the range of 150-0 mM ammonium sulfate. For further purification, HALP-SHIS was passed through on a hydroxylapatite(PENTAX)column equilibrated with 100 mM phosphate buffer(pH 7.2). Purified HALP-SHIS was migrated in the SDS-PAGE to two positions corresponding to molecular masses of 37 kD and 39 kD. On western blot analysis with a polyclonal antibody against crude HALP-SHIS,the both bands were blotted.Determining the NH₂-terminal amino acid sequence of HALP-SHIS is being carried after SDS-PAGE and transfer onto PVDF membrane. We will be discussed a possible mechanism for complement and whether HALP-SHIS protein is similar to complement of vertebrates.

07-052

SUBTRACTIVE cDNA CLONING OF *HYPHANTRIA CUNEA* (LEPIDOPTERA: ARCTIIDAE) LARVAL GENES INDUCED BY INJECTION OF *ESCHERICHIA COLI*

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cDNA library, induced by injection of *E. coli* to the larvae of the fall webworm, *Hyphantria cunea*, was subtracted with uninduced mRNA of the larvae. The total RNA was isolated from the immunized larvae and cDNA library using Uni-ZAP XR vector was constructed. This library was infected to the host cells (XL1-Blue MRF') with helper phages (VCSM13), and single strand DNA phages were excised by in vivo excision protocol. From these phages, single strand DNA (pBluescript SK(-) phagemid) was isolated and twice subtracted with 10-fold biotin-labelled mRNA from the unimmunized larvae. By PCR reaction with subtracted single strand DNA as template, the cDNA insert was amplified and cloned to the plasmid. DNA sequences of some clones revealed sequence homology to attacin, cecropin, protease inhibitors. These clones were characterized by cDNA cloning, northern hybridization and southern hybridization.

07-054

CARBOXYLESTERASES OF *BOMBYX MORI* INDUCED BY INFECTION OF *E. COLI*,

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There has been known many proteins, including anti-bacterial peptides, that are induced by bacterial infections. We found that ones of those induced proteins are carboxylesterases (CEs) in *Bombyx mori*. The CEs were induced after 24 hours not only by infection of *E. coli* but injection of lipopoly saccharides. The characteristics of the induced CE are different from juvenile hormone esterases or general CEs with no induction in terms of isoelectric points of the molecule. That is, pI values of the induced CE isozymes are approximately 6.0 in contrast with those of JHE (4.9) and general CE isozyme (4.8). The characteristics and function of those CEs are under investigation.

07-055**ISOLATION AND CHARACTERIZATION OF A NOVEL TYPE OF ANTIBACTERIAL PEPTIDE FROM THE SILKWORM, *BOMBYX MORI***

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A novel antibacterial peptide that shows antibacterial activity against *Staphylococcus aureus* was isolated from the immunized hemolymph of the lepidopteran, *B. mori*. This peptide (named moricin) consisted of 42 amino acids and was highly basic. Moricin indicated no significant similarity with other antibacterial peptides. Moricin showed antibacterial activity against several Gram-negative and -positive bacteria and had a higher activity against Gram-positives than cecropin B₁, an antibacterial peptide of *B. mori*. Moricin was inducible by bacterial injection. These results suggest that moricin is a novel member of antibacterial peptides and is responsible for the antibacterial activity in *B. mori* against Gram-positive bacteria.

The effects of moricin on bacterial and liposomal membranes indicated that a target of moricin is the bacterial cytoplasmic membrane. The results also suggest that the N-terminal portion of moricin, containing a predicted α -helix, is responsible for an increasing in the membrane permeability.

To obtain a large amount of this peptide, a production system in *Escherichia coli* was also constructed.

Section 8

Genetics and Evolutionary Entomology

08-002

ORIGINS AND GENE FLOW IN COLORADO
POTATO BEETLE

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Geographic structure and gene flow were estimated in Colorado potato beetles from native and agricultural habitats using protein electrophoresis and mtDNA. Using electrophoretic data, estimates of gene flow, N_{em} (calculated from theta) were 3.8 and 14.5 for populations from native and crop habitats, respectively, over the same geographic scale (approx. 500 km). Populations were sampled over time to examine year-to-year variation in geographic structure and assess genetic equilibrium. The estimate of N_{em} from mtDNA analysis of 10 populations from across the U.S. was 0.7. Genetic data is used to shed light on several hypotheses concerning the shift from native hosts to potato.

08-001

GENETIC STRUCTURE IN FRAGMENTED RELICTUAL POPULATIONS: HINTS FROM *DOLICHOPODA* CAVE CRICKETS

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Evolutionary divergence and speciation in *Dolichopoda* cave crickets is essentially a geographic process. The model assuming fragmentation of a widespread epigean species and isolation of small populations in cave refugia, where divergence is mainly driven by genetic drift, appears suitable for *Dolichopoda*, where a metapopulation structure is often revealed.

The degrees of genetic structuring, as measured by Wright's F_{st} , were calculated for multiple combinations of populations at different geographic scales. F_{st} and m (migration rate) values were calculated on the basis of polymorphic loci and estimated population sizes on different groups of *Dolichopoda* populations by considering geographic windows progressively smaller. Comparisons among different geographical windows indicate that F_{st} and m values depend on the proximity between populations and the habitat structure surrounding caves. As expected, m values increase as the window scale decreases, and migration is actually detectable only between very proximate populations. At this scale, the extent of gene flow varies between populations sampled in areas with different bioclimatic conditions. Groups of populations from coastal mediterranean habitats are genetically more structured than those living in montane mesophilous forest, as supported by records of *Dolichopoda* in pitfall traps.

Population structure shapes the patterns of geographic variation of allozyme frequencies. Improvement of geostatistical tools to represent and analyze geographic variation of multivariate sets of characters helps to clarify, through the comparative study of patterns of variation at different geographic scales, the shaping, and the tempo and mode, of species formation. By this way, allopatric populations may be represented as genetic landscapes in the geographical space.

08-003

INTRON SIZE VARIATION TRACES THE WORLD-WIDE COLONISATION HISTORY OF THE MEDFLY *CERATITIS CAPITATA*

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The 1st intron of the *Adh1* gene is unexpectedly long in *C. capitata* compared to that of *D. melanogaster*, and contains a sequence homologous to the *mariner* element of *Drosophila mauritiana*.

Using PCR, the *Adh1* first intron was isolated from 27 populations throughout the range of *C. capitata*. Eighteen size variants were found, ranging from 1400 bp to 3450 bp, each falling into one of four distinct size categories. The number of size variants is highest in populations from within the supposed ancestral range of *C. capitata*, i.e. Kenya. The degree of polymorphism declines rapidly towards the periphery of the global distribution, where one finds a subset of two or three size variants. The same trend of decreasing variation from the putative source area to the derived populations has previously been found using RAPD and allozyme methods in wild populations and laboratory strains of *C. capitata*. The intron variant frequency data was used to construct dendrograms to infer the relationships between the different wild populations.

The extent of homology between the different intron variants was analysed by means of their restriction maps. The four size categories can be explained by three major differences in their maps. The small intron category lack the *mariner*-like element. The medium and the long and very long intron categories all possess the *mariner*-like element but differ in the presence and size of an insertion/deletion in the second half of the intron. Variation within each category appears to be due to small insertions/deletions throughout the intron. Each of the two widely spread intron size variants were found to be identical in all the peripheral populations analysed. In the ancestral populations, however, restriction site polymorphisms were found in the variants. This further supports the derived nature of the peripheral populations and emphasises the restricted level of variation within and between these populations.

08-004

INVASION GENETICS AND PHYLOGEOGRAPHY OF THE MEDITERRANEAN FRUIT FLY: DNA SEQUENCES FROM MULTIPLE NUCLEAR LOCI

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Biological invasions generally start from low initial population sizes, leading to reduced genetic variation in nuclear and especially mitochondrial DNA. Consequently, genetic approaches to invasion history and population structure of many exotic pests and pathogens are difficult. For example, during invasions over the past century populations of the Mediterranean fruit fly, *Ceratitis capitata*, have lost 60 to 70 % of ancestral genetic variation at nuclear loci and a staggering 96 % of variation in mitochondrial DNA. This result has thwarted the use of genetic methods to understand the invasion history of Medflies. We present a new approach to invasion genetics that measures novel DNA sequence variation within introns from multiple nuclear loci. Not only are such loci variable, even within recent populations in both California and Hawaii (USA), but also they suggest that the number of emigration events from Africa were relatively few. Intron variation in species such as the medfly allows high resolution genetic characterization of invading populations and the potential to identify the numbers of colonization events and their sources.

08-005

EVIDENCE, USING GENETIC MARKERS, OF RESTRICTED GENE FLOW (MIGRATION) WITHIN AND BETWEEN BRITISH POPULATIONS OF THE DAMSON-HOP APHID *PHORODON HUMULI* SCHRANK (HEMIPTERA : APHIDIDAE)

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The damson-hop aphid, *Phorodon humuli* Schrank, is a serious pest of hops in England. It is holocyclic (with obligatory sexual phase) and host alternating. From suction trap data, *P. humuli* aerial densities are known to be highest in the main hop growing regions viz. Hereford (mid-west) and Kent (south-east), some 260 km. apart. The aphid is now resistant to several insecticides. This is, in part, conferred by elevated (carboxyl)esterase activity, ranging from low in susceptible to high in very resistant strains.

Enzyme markers separated by gel electrophoresis, including carboxylesterase, have been used to examine the degree of genetic heterogeneity among *P. humuli* subpopulations on both its hosts (*Prunus* spp. (primary host) and hop, *Humulus lupulus* (secondary host)). The esterase data revealed heterogeneity among subpopulations, with a higher mean frequency of elevated esterase genotypes in the hop growing regions. Similarly, allele and genotype frequencies for another enzyme (6PGD) were also heterogeneous, even between subpopulations sampled at less than 30 km. apart in each of the two regions surveyed (especially from *Prunus*), whilst allele and genotype frequencies sometimes remained stable over a number of summers. Also, 6PGD genotype frequencies were sometimes in, or close to, Hardy-Weinberg expectations, even for parthenogenetically-reproducing aphids infesting the secondary host, hops. These data sets suggest that there is restricted inter-population gene flow, and hence migration, in this species. Presently, DNA data (RAPD) are being gathered to reveal the 'fine-grained' genetic structure of populations. The data are discussed in the light of previous studies on the population genetics of aphids using molecular markers (allozymes and DNA).

08-006

MOLECULAR MARKERS LINKED TO LIFE CYCLE VARIATION IN THE CEREAL APHID *RHOPALOSIPHUM PADI* L. (HOMOPTERA: APHIDIDAE)J.C. Simon, D. Martinez-Torres¹, A. Moya¹, A. Latorre¹, P.D.N. Hebert²

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Although most aphids reproduce by cyclical parthenogenesis, some species show variation in their mode of reproduction leading to life cycle polymorphism. Among the latter, *Rhopalosiphum padi* is particularly interesting since its populations may be a mixture of holocyclic clones (cyclical parthenogens), anholocyclic clones (obligate parthenogens) and androcyclic clones (producing some males in addition to parthenogenetic females). In this study, we analyzed mitochondrial DNA, RAPD and SCAR markers in *R. padi* as well as plasmid DNA of its endosymbiont to determine the genetic relationships between its sexual and asexual populations and to develop molecular tools for the characterization of its life cycle variants.

A strong relationship between life cycle and mtDNA as well as plasmid DNA was found: all holocyclic clones carried haplotypes II or III while most of the anholocyclic or androcyclic clones were haplotype I. The restriction site analysis on these two maternally inherited molecules suggested an ancient origin of asexual populations in *R. padi* followed with interrupted gene flow with sexuals. However, evidence of occasional gene flow between sexual and asexual lineages, presumably mediated by androcyclic males, was also provided and its consequences on the evolution of anholocyclic populations were evaluated. Lastly, we identified a RAPD marker linked to the life cycle variation in *R. padi* based on a bulk segregant analysis on F2 progeny of crosses between holocyclic and androcyclic clones. This marker was converted into a SCAR and its first applications on natural populations are presented.

08-007

CHROMOSOMAL LOCALIZATION OF A HIGHLY REPEATED *ECORI* FRAGMENT IN *MEGOURA VICIAE* (HOMOPTERA, APHIDIDAE) BY NICK TRANSLATION AND *IN SITU* HYBRIDIZATION.

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To investigate the genome of the aphid *Megoura viciae* at the molecular level, we studied total DNA by agarose gel electrophoresis after cleavage with different restriction endonucleases. *EcoRI* digestion produced a highly repeated DNA fragment, about 600 bp long. The contribution of this *EcoRI* element to the total genome of *M. viciae* has been estimated at 6% by means of densitometric scanning of photographs of agarose gels. The chromosomal localization of this satellite DNA, investigated by fluorescent *in situ* hybridization (FISH), constantly showed one main and two secondary fluorescent bands on the X chromosome, all corresponding to C-positive heterochromatic areas. These results are in full accordance with the data obtained by *in situ* nick translation experiments carried out after *EcoRI* digestion, and clearly demonstrate that the major fraction of *M. viciae* heterochromatin, which consists of *EcoRI* fragments, is essentially located on the X chromosome. Our data represent a substantial contribution to a better understanding of genome organization in *M. viciae*. Moreover, the utilization of the *EcoRI* restriction fragment as a molecular probe to be tested on DNAs obtained from several species, may represent a valuable tool for investigating taxonomic and evolutionary relationship in aphids.

08-008

INVESTIGATION OF THE UNDERLYING CAUSES OF HOST-BASED GENETIC VARIATION IN ASEXUAL POPULATIONS OF THE GRAIN APHID SITOBION AVENAE (F.) (HEMIPTERA: APHIDIDAE)

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The grain aphid *Sitobion avenae* (F.) was collected from winter wheat and adjacent cocksfoot in southern Britain. Genetic variation between individuals was then investigated using multilocus probes. Aphids caught on wheat and cocksfoot in spring had highly characteristic genetic profiles, indicating host-based genetic similarity. However, this host-based genetic structuring became less distinct over time, and may have been as a result of local movement of aphids from cocksfoot to wheat.

In choice chamber experiments, winged summer forms preferred to colonise wheat rather than cocksfoot. Furthermore, those aphids collected from cocksfoot showed a lower preference for wheat than those collected from wheat. In performance experiments wingless forms generally had higher weight, fecundity and survival when reared on their host of origin. In both experiments, rearing aphids on alternate hosts over successive generations had a significant conditioning influence. Overall, the study reveals host-based genetic variation in a largely asexual species, while the evidence for clonal variation in both preference and performance confirms the existence of two processes by which this genetic stratification can be generated and maintained.

08-010

WHAT IS AN APHID SPECIES?

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The conceptual and practical problems of defining and recognising aphid species, and especially aphid pest species, will be discussed in the light of recent advances in our knowledge of their population structure, life cycle variations and interactions with host plants. A distinction is drawn between the conception of species as functional, interactive, co-evolving units of life, and the working definition of a species, that attempts the seemingly impossible task of imposing static limits on essentially dynamic situations.

With the advent of molecular tools that will enable us to study, and perhaps eventually to predict, changes in the genetic structure of aphid populations, how useful is the species "tag", and how far can we go with the recognition of taxa below the level of the species?

08-009

GENETIC DIFFERENTIATION, SPECIATION AND HOST PREFERENCES IN APHIDS OF THE *HYALOPTERUS PRUNI* COMPLEX (HOMOPTERA, APHIDOIDEA)

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Previous isozyme studies (Spampinato *et al.*, 1988) have shown that the *Hyalopterus pruni* complex includes at least three distinct biological species: *H.pruni sensu stricto* (primary host plants, plum and apricot trees), *H.amygdali* A (primary host, almond and occasionally peach trees), and *H.amygdali* B (primary host, peach and occasionally almond trees). Further research at 30 enzyme loci on various European populations of the *H.pruni* complex, both sympatric and allopatric, have allowed: *i*) to estimate their genetic divergence and evolutionary relationships; *ii*) to identify by molecular markers the three species and their possible hybrids, and to assess reproductive isolation; *iii*) to investigate host preferences in the field.

H.pruni and *H.amygdali* A, which show the lowest genetic divergence ($D_{Nei}=0.12$) were never found to share the primary host; on the other hand, *H.amygdali* A and *H.amygdali* B, the two genetically most differentiated ($D_{Nei}=0.32$), show a more similar trophic niche, as they share the same primary host wherever the preferential one is lacking. Moreover, the few interspecific F_1 hybrids detected in the field were in mixed populations of *H.amygdali* A and B living on the same primary host (peach trees). No gene exchange was observed between any of the three species (lack of backcross, recombinant or introgressed genotypes); their reproductive isolation is therefore demonstrated. The possible speciation processes occurred in this complex are discussed.

08-011

THE COEVOLUTION OF APHIDS (HOMOPTERA: APHIDOIDEA) AND THEIR BACTERIAL ENDOSYMBIONTS (*BUCHNERA APHIDICOLA*): MOLECULAR PHYLOGENETIC APPROACHES

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Molecular phylogenetic analyses indicate that aphids and their bacterial endosymbionts have cospeciated since the time of an infection of a common ancestor of extant members of the Aphidoidea, over 100 million years ago. Studies of the tryptophan biosynthetic pathway within the endosymbionts have demonstrated that the genes for the rate-limiting step are amplified on plasmids within the Aphididae, allowing overproduction of this nutrient for the aphid host. Phylogenies derived independently using sequences of genes borne on these plasmids, on the endosymbiont chromosome, and on the aphid mitochondrial chromosome are similar, supporting the hypothesis that plasmid genes were derived from the chromosome and that all genomes are vertically transmitted.

Molecular phylogenetic studies of the Aphidoidea and of major groups within the Aphididae are also presented.

08-012

MICROSATELLITE TRACKING OF PHYLOGENY AND RECENT EVOLUTION OF *SITOBION* (HEMIPTERA: APHIDIDAE)

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Sitobion is a genus of about 75 species, of which almost half feed on Gramineae, often causing economic damage. Two species of probable East Asian origin, *S. miscanthi* and *S. nr fragariae*, occur on grasses in Australia. *S. miscanthi* is thought to have reached Australia naturally, prior to European settlement, but *S. nr fragariae* has been recognised here only since the 1960s. Many possible synonymies of the grass-feeding Asian *Sitobion* spp. have been proposed. Further confusion arises from the existence in Australia of at least five chromosomal races of *S. miscanthi*.

We have used microsatellite DNA analysis to investigate the phylogeny of *Sitobion* spp. feeding on Gramineae, and to examine the local evolution of the two species occurring in Australia. Both are assumed to be continuously parthenogenetic here, and this hypothesis is also tested. The work is the first to examine the genetics and evolution of parthenogenetic invading species using microsatellites.

Four microsatellite loci have been scored for a range of geographically separated Australian populations of *Sitobion*, as well as non-Australian *S. avenae*, *S. fragariae*, and *S. akebiae*. Along with sequence data from single locus nuclear genes in some species from hosts other than grasses, these data enable us to present a phylogeny of the species considered. There seems to have been a single successful invasion of *S. nr fragariae* into Australia, while *S. miscanthi* is represented by survivors from two separate migrations. One of these has given rise to the 2n=18 and 19 chromosomal races, and the other to the 2n=17, 20 and 21 races. The gene pool within Australia is very limited, and there is no evidence of sexual reproduction by either species, in contrast to the situation in Asian and European populations.

08-014

AUTUMN COLOURS: A ROLE FOR APHIDS?

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A crude but objective scoring of the autumn colours of deciduous trees in North America and Europe reveals independent significant positive correlations of the red and (more strongly) the yellow of trees with incidence per tree species of single primary host aphid species. Although coefficients are always positive, correlations of the colours with aphid parasitism specific only within genus, or within 2, 3, or >3 genera, are in no case significant. Applying phylogenetic regression (A. Grafen) and controlling for several possible confounding variables of tree characteristics and geography hardly changes the results.

Aphids use colour vision to find hosts. Primary hosts are ancient associates for most aphids so that aphids, especially those host-specific, will have damaged hosts throughout long co-evolution. This will have occurred through conveyed virus as well as directly through feeding.

It is postulated that trees synthesise pigments in leaves about to be shed because they serve as handicap demonstrations of tree vigour. They deter so far as possible aphids from landing and egg-laying: The 'message' is that a strong tree will mount a strong defence, probably chemical, against aphid feeding in spring.

Other insect groups possessing colour vision may be involved and this is especially likely for reds for which our correlation is weak. Work is needed to test all the above suggestions.

08-013

THE PHYLOGENETICS AND HISTORICAL BIOLOGY OF THE APHID TRIBE CERATAPHIDINI (HOMOPTERA: APHIDOIDEA: HORMAPHIDIDAE)

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To elucidate the evolution of several important traits within aphids I have been studying the molecular phylogenetics of the Cerataphidini. Using mtDNA sequence data I have estimated the phylogeny for over 35 species of the Cerataphidini. I have used this phylogeny to examine (1) the evolution of sterile soldiers on both the primary and the secondary host, (2) the evolutionary reduction of complex life cycles, (3) the evolutionary replacement of one endosymbiont by another, and (4) the evolution of gall morphology. In addition, the DNA sequence data has proved useful in determining the life cycles of tropical aphid species that have been otherwise difficult to study.

08-015

THE EVOLUTIONARY HISTORY OF APHIDS AND A HYPOTHESIS ON THE COEVOLUTION OF APHIDS AND PLANTS

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Fossil aphids, including several specimens recently found in amber from the Cretaceous and the Lower Tertiary, not yet described, and comparison between the aphid fauna of the past and the recent fauna, indicate two major faunal changes, which are confirmed by the new findings. The first change happened at or close to the Cretaceous-Tertiary boundary, 50-70 million years ago. The composition of the faunas, respectively about 75-80 million old and about 35-45 million years old, are described and compared. Most aphid families present in the Cretaceous became extinct before the Lower Tertiary, the period, in which the Baltic amber was produced. In the Lower Tertiary most recent families were present, but the families Aphididae and Lachnidae were much less rich in species than they are today. These changes are described as consequences of changes of the flora, from gymnosperms to angiosperms, and from predominantly woody angiosperms to herbs, especially herbs belonging to the plant families Poaceae and Asteraceae, which became rich in species during the Upper Tertiary and now contain the hosts of most aphids.

Among new species of Cretaceous age are representatives of the families Canadaphididae and Palaeoaphididae, previously only known from Canadian amber deposited in a secondary site. Two additional specimens of *Canadaphis carpenteri* Essig, 1937, show a long rostrum, not visible in the holotype. The presence of siphuncular pores shows that Canadaphidoidea is a synonym of Aphidoidea. Among the representatives of Palaeoaphididae is a member of a new genus with long rostrum and bilobed posterior end of the abdomen. Instead of siphuncular pores it carries small cuticular fields with very small pale spots.

08-016

TWENTIETH-CENTURY MORPHOMETRICS
What We Know, What We Don't

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At the core of contemporary morphometrics, the quantitative study of biological shape variation, is a synthesis of two originally divergent methodological styles. Conventional multivariate analysis of covariances or correlations among multiple measures of shape or size sometimes results in optimal statistical descriptions of group differences, but this approach ("multivariate morphometrics") cannot properly exploit the origin of our data in measurements on specimens or pictures. Another tactic much more in the spirit of observational biology emphasizes the direct visualization of changes in biological form by comparative drawings upon which relevant measurements or deformation grids are sketched. But it proved very difficult to convey the variation and covariation of aspects of form, as distinct from pairwise contrasts, in diagrams like these. It has thus been clear for decades that some methodological synthesis was urgently needed.

The solutions that have been worked out for this problem of synthesis differ for the different data structures typical in our field. All spring from new, more careful consideration of the way "size and shape data" arise in quantitative biology. One new abstraction is crucial: the space of all shape measures, for data of the relevant type (weights, landmarks, etc.), "in the vicinity of an average form." The structure of this space varies according to the nature of the data. A suite of net size measures, such as overall organ weights or volumes, is approached via the study of all allometric ratios (log-linear combinations). Modern variants of principal-components analysis are well-suited to investigations of this sort. When data arise as a set of measured distances, whether or not between landmarks, we may exploit the fact that ratios of distances are, themselves, shape measures. The special role of geometric shape as distinct from allometric shape emerges here because of its power for generating new descriptions of findings. Finally, we have just recently designed a new, startlingly powerful version of multivariate analysis for data that are coordinates of landmark points. Here, one single analysis combines statistics and graphical display of findings for all possible shape analyses simultaneously.

08-018

PHYLOGENETIC PATTERNS AND MORPHOMETRIC
DISTANCE IN LYGUS BUGS

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Lygus bugs (Heteroptera:Miridae) are plant-feeding insects causing economic losses to many crops in the Northern Hemisphere. Consequently *Lygus* species are the subject of much basic and applied research. However, they are taxonomically difficult, due to subtle character differences and character variability. This genus has recently been subjected to a broad systematic analysis involving traditional morphological characters, morphometric and molecular data and a range of phylogenetic methods in order to resolve the relationships among the species. This paper compares the results from different approaches to determining morphological distance among *Lygus* species using different data sources and discusses the results of different methods of comparison, particularly between a partially resolved molecular phylogeny and phylogenies derived from morphometric sources.

08-017

EVOLUTION OF WING FORM AND VENATION IN THE *DROSOPHILA*
MELANOGASTER SUBGROUP (DIPTERA, DROSOPHILIDAE)

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Wings of *Drosophila* have been characterized by fourteen homologous landmarks on both sexes of the eighth species of the *melanogaster* subgroup, the three mitochondrial types of *D. simulans* (sil, sill and silli) as well as some interspecific F1 hybrids. Geometric morphometric analyses were done using Procrustes superimposition and Thin plate splines partial and relative warps. Species and sexes were separated by principal components and discriminant analyses. The overall misclassification was as low as 2.8 % and dealt mostly with *D. melanogaster* and *D. erecta*. The most important shape divergence was encountered within the monophyletic group build by *D. simulans*, *D. mauritiana* and *D. sechellia*. Within these three species, evolution of wing shape supports a sister-group relationships between *D. mauritiana* and *D. simulans*. These analyses helped to reveal hidden patterns of shape changes, which encompass clearly the classical longitudinal shift of the R1 apex. These shape patterns are analysed in relationships with intra- and interspecific allometries and in the light of wing compartments.

08-019

MORPHOMETRICS AND THE ANALYSIS OF
DEVELOPMENTAL PROCESSES

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Morphology is the product of developmental processes, and differences in morphologies are due to differences in those processes. Although it is possible to define arbitrary functions that accurately describe the relationship between two morphological traits, and arbitrary discriminant functions that can accurately distinguish between different morphologies, in order to understand the development and evolution of a character suite the descriptive model should also capture the essence of the processes that gave rise to those relationships and differences.
The morphometric relationship between two characters can be interpreted in terms of differences in the parameters of a developmental model that can produce those characters. Two such analyses will be discussed. (1) The allometric relations among body parts in holometabolous insects are seldom linear. Most often they are curvilinear or broken and discontinuous. We have modeled the growth of imaginal structures and examined the consequences of inter-disk competition in the closed environment of the pre-pupa on the growth of disks. We show that non-linear and complex allometries are the consequence of normal growth processes of holometabolous insects. (2) Genetic crosses, experimental perturbation methods, and theoretical models, have revealed that the control mechanisms that regulate the development of lepidopteran color patterns are highly localized. Morphometric analysis of individual variation likewise reveals an almost complete lack of correlated variation among pattern elements. Together, the morphometric and experimental data reveal a highly flexible developmental system and help explain the great evolutionary radiation of color patterns and the ease with which adaptive patterns and accurate mimicry can evolve.

08-020

GROWTH STUDIES IN HEMIMETABOLOUS INSECTS: ANALYSIS OF ONTOGENETIC VARIATION AND CONSTRAINTS

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Hemimetabolous insects, especially those with a constant number of instars, provide an excellent opportunity to study ontogenetic variation. The molts are distinct ontogenetic stages corresponding among individuals and may be considered homologous across species for comparative studies. Longitudinal growth data are fairly easy to obtain by collecting exuviae from individually reared insects.

I present results of such an analysis in the water strider *Limnoporus canaliculatus* (Heteroptera: Gerridae). A new statistical technique made it possible to study the covariation among characters simultaneously within and across instars. The underlying model assumes that one common pattern not only underlies the variation within instars, but also the covariation among stages. The data from *Limnoporus* conform fairly well to this assumption.

Analyses of variation in instar-specific size measurements (cumulative data) differ from analyses of growth increments. Ontogenetic trajectories for cumulative size are strongly constrained and show only a few patterns of variation, which also have been found in studies of mammals and birds; these result from the part-whole relationships inherent in cumulative data, but do not reflect developmental processes in the study organisms. In contrast, incremental data show very little ontogenetic integration or constraints; this flexibility possibly accounts for the substantial interspecific variation of growth increments in the genus *Limnoporus*.

For a more complete understanding of the evolution of ontogenies, quantitative genetic experiments and field studies of life histories, the ecological aspect of ontogeny, should be combined with this morphometric approach.

08-021

INSTAR DETERMINATION USING FINITE MIXTURE ANALYSIS: Dyar's rule meets maximum likelihood

Bernard D. Flury and Cameron R. Currie

Size measurements, e.g., head capsule widths, have been used traditionally to identify instars in populations of insects. However, this is a difficult problem if the size distributions of different instars overlap. Ad-hoc methods are usually applied, such as counting the number of peaks in a histogram, or approximation of a multimodal histogram by a combination of several normal densities using least squares (McClellan and Logan 1994, Environ. Entomol. 23: 248-253, 1994). A more sophisticated approach is given by the methodology of finite mixture analysis, which allows to estimate the parameters of distributions that consist of homogeneous sub-populations mixed in unknown proportions. We give a non-technical introduction to finite mixtures and their estimation by maximum likelihood, and illustrate the technique using data measured on several insects with diverse life history strategies, such as forest defoliators and wood-boring beetles.

08-022

MORPHOMETRIC ANALYSES FOR FUNCTIONAL MORPHOLOGY

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Morphological description is a necessary part of biomechanical or functional studies. However, it is not always obvious which morphological features are functionally relevant in a particular case; mechanical phenomena can scale in a nonintuitive or even a counterintuitive way with form. For example, sometimes large differences in size or shape are without functional correlation and sometimes even a small anatomical change will make a huge difference.

Can one look at the size and shape of an insect antenna and know anything *a priori* about its capacity to intercept chemical signals from the air? Using physical models based on the geometry of *Bombyx mori* antennae, an engineering technique was used to estimate the mass transfer coefficients of individual sensilla (the rate at which material can be exchanged between a structure and its surrounding environment). The results do scale with surface area as expected. However, the mass transfer coefficients are also sensitive to the proximity of other hairs. Thus another anatomical feature, the distance between hairs, is also functionally significant. The magnitude of this influence depends both on the speed of the air flow and the spatial distribution of the chemical signal.

08-023

SCALING SIZE TO FITNESS

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Size at metamorphosis and adult size in mosquitoes is generally reduced if larvae experience higher temperatures, higher density, or reduced resources. The number of ovarioles in adult females increases monotonically with increased female mass and the number of eggs per batch, clutch, or ovarian cycle reflects this relationship. Size also affects adult survivorship. Survivorship may be a linear, exponential, or parabolic function of size. Individual fitness expressed as expected lifetime production of offspring is a product of survivorship and fecundity. The relative magnitude of size-dependent fecundity and size-dependent survivorship then determine whether fitness scales to a linear, logarithmic, exponential, or parabolic function.

08-024

EVOLUTION AND PHYLOGENY OF *WOLBACHIA*

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Wolbachia are rickettsial bacteria that are widespread in arthropods and cause a variety of reproductive alterations in their hosts, including reproductive incompatibility, parthenogenesis and feminization of genetic males. These reproductive alterations are selectively advantageous for the microorganisms and have important evolutionary implications for evolution of their hosts. A phylogenetic study of *Wolbachia* using 16S ribosomal and protein coding regions reveals that these bacteria are diverse and undergo extensive horizontal transfer between different arthropod taxa. Phylogenetic data indicate that transfer between parasitic wasps and their insect hosts may occur. Parthenogenesis induction by *Wolbachia* appears to have evolved several times independently. Infections with 2 different strains of *Wolbachia* occur in some arthropod species. Finally, there is accumulating evidence that incompatibility *Wolbachia* may be a factor promoting reproductive isolation and rapid speciation in arthropods.

08-026

DIVERSITY OF FEMINIZING *WOLBACHIA* IN ISOPODS

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Wolbachia are strictly endocellular, vertically transmitted bacteria associated with insects and crustaceans. They are responsible for feminization, cytoplasmic incompatibility (CI) or parthenogenesis in their hosts, which enable them to increase their transmission in the host populations. By using PCR diagnostic primers we have found *Wolbachia* in 20 isopods : *Wolbachia* is widespread in Oniscidea occurring in all families and is also present in Asellota and Flabellifera. The taxonomic status of these microorganisms has been defined by sequencing parts of their 16S rDNA and *ftsZ* genes. The bacteria from Oniscidea hosts show a very low divergence. Even with the more variable *ftsZ* gene, some *Wolbachia* sequences are identical in host species belonging to different families. All these bacteria form a monophyletic group which belong to the B subdivision of *Wolbachia* whereas bacteria from Asellota and Flabellifera are more close to parthenogenesis and CI *Wolbachia* of insects. The lack of concordance between bacterial and host phylogenies indicates that the crustacean-infecting *Wolbachia* have undergone horizontal transfer amongst isopod lineages and may be between insects and crustaceans. Other results including success of microinjections of bacteria are consistent with the view that horizontal transfer has been predominant in the modern distribution of *Wolbachia*. Uninfected males and females of woodlice were inoculated by endosymbionts from 6 other species. Most of the recipient males were feminized by the inoculated bacteria showing their ability to induce feminization, symptom only known in crustaceans. Foreign *Wolbachia* were also able to live in most recipient females. However, the results varied from a successful transmission to the offspring to a detrimental effect on female fecundity. In the first case, the highly female-biased progenies always occurred when the donor and the recipient are sibling species. These results show that horizontal interspecific transfers can contribute significantly to the wide distribution of *Wolbachia* within terrestrial isopods, even if their success is not predictable.

08-025

PARTHENOGENESIS *WOLBACHIA* IN HYMENOPTERA

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Female wasps infected with parthenogenesis *Wolbachia* are capable of producing daughters without mating. This type of infection has now been found in approximately 20 species of Hymenoptera spread over at least 6 families. Many more cases are suspected. The *Wolbachia* allows unfertilized eggs to become females by influencing the first mitotic division in the egg. The presence of these *Wolbachia* is associated with an aborted first mitotic division resulting in a fusion of the two sets of identical mitotic chromosomes. Thus the resulting diploid and therefore female offspring is 100% homozygous. In most species studied all individuals of a species are infected, but in a number of *Trichogramma* populations both infected and not-infected (i.e. sexual) wasps are found together. In such populations males will mate with the infected females and their sperm will be used for the fertilization of eggs. Such fertilized eggs become infected diploid females, whose genetic make-up consists of one set of chromosomes from the father and one from the mother. In such mixed populations the infected fraction of the population is not genetically isolated from the sexual forms. The infection with *Wolbachia* appears to have a substantial negative effect on the offspring production in infected females from such mixed populations. These negative effects on offspring production are not evident in those species where the infection has gone to fixation. The relationship between the parthenogenesis and incompatibility *Wolbachia* is not quite clear. Using phylogenetic methods it can show that the *Wolbachia* are divided in two groups, both groups contain incompatibility and parthenogenesis *Wolbachia*. Contrary to the situation in incompatibility infections where representatives of both groups can be found in one infected individual not double infections have been found to date.

08-027

TOWARDS A BETTER UNDERSTANDING OF THE MOLECULAR BASIS OF *WOLBACHIA*-MEDIATED CYTOPLASMIC INCOMPATIBILITY

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The phenomenon of *Wolbachia*-mediated cytoplasmic incompatibility has been known from insects for many years. However, an understanding of the mechanism of how *Wolbachia* is able to disrupt early fertilization events in insects has yet to be determined. We report on our efforts to unravel this system, including a method for *in vitro* cultivation of *Wolbachia* and characterization of the dominant *Wolbachia* protein found in insect testes. We provide indirect evidence that this protein may be functionally significant with regard to the expression of cytoplasmic incompatibility.

08-028

CYTOGENETIC EFFECTS OF INCOMPATIBILITY
WOLBACHIA

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ABSTRACT NOT RECEIVED

08-029

DISTRIBUTION OF WOLBACHIA AND CYTOPLASMIC
INCOMPATIBILITY IN MITES

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Wolbachia are a group maternally inherited bacteria that manipulate normal reproduction of their arthropod host to enhance their own transmission. They are widespread in insects, but are little studied in other arthropods except for a few isopods. *Wolbachia* are implicated as the cause of parthenogenesis in parasitic wasps, feminization of genetic males into functional females in isopods, reproductive isolation (cytoplasmic incompatibility) between and within many species (insects and an isopod) and yet others do not seem to have a noticeable effect on their host. Despite their widespread distribution in insects and various effects they have on their host, they form a monophyletic group of closely related group of intracellular bacteria based on 16S and *ftsZ* DNA sequences.

Mites (Order Acari) often show postzygotic reproductive isolation between populations and closely related species, but the genetic basis is not well understood. Presence of cytoplasmic incompatibility *Wolbachia* resulting in reduced crossability could be one explanation. Here I report on the widespread occurrence of *Wolbachia* in mites based on a PCR assay with primers specific for *Wolbachia ftsZ* gene, and their effect on the mite host. In the spider mite, *Tetranychus urticae*, *Wolbachia* cause cytoplasmic incompatibility. Crosses between infected and uninfected (antibiotic treated) mites resulted in increased embryo mortality among offspring. As in Hymenoptera, this spider mite is haplodiploid: females develop from fertilized eggs and males from unfertilized eggs. Cytoplasmic incompatibility in parasitic wasps typically results in all male offspring, because of paternal chromosome elimination which renders the egg haploid. Unlike parasitic wasps, cytoplasmic incompatibility in this spider mite resulted in mortality among fertilized eggs: the number of sons (unfertilized eggs) is not affected. The widespread presence of *Wolbachia* in mites may very well parallel the situation in insects. Moreover, predatory and parasitic mites may play a role in horizontal transmission of *Wolbachia* not only among mites but also among insects.

08-030

SPREAD OF INCOMPATIBILITY WOLBACHIA THROUGH
POPULATIONS

A.A. Hoffmann

ABSTRACT NOT RECEIVED

08-031

COMPARISON OF MOLECULAR AND REPRODUCTIVE
VARIABILITY ASSOCIATED TO CYTOPLASMIC GENOMES IN
THE MOSQUITO, *CULEX PIPPIENS*

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Bacteria of the genus *Wolbachia* are responsible for reproductive incompatibilities between *Culex pipiens* mosquitoes assumed to harbour different *Wolbachia* strains. Past work has suggested the existence of considerable polymorphism with regards to incompatibility properties in populations of this mosquito. So far, it has been found that in other host species (*Drosophila simulans*, *Aedes albopictus* and *Nasonia vitripennis*) incompatible infected strains harbour different *Wolbachia* strains that can be distinguished by sequences of their 16S rRNA and *ftsZ* protein gene. To investigate the presence of similar multiple infections in mosquitoes we have sequenced a fragment of the *ftsZ* protein gene of symbionts from strains of various geographic origin, taxonomic (subspecies) affiliation, and with different compatibility relationships. We found no variation. We have also sequenced the control region of *Culex* mtDNA in these strains and found a low level of variation. This suggests that cytoplasmic genomes of most *Culex pipiens* mosquitoes have a relatively recent common ancestor, and that differentiation of incompatibility types in this species has occurred since this recent common ancestor. However, the exact factors responsible for this differentiation are still unknown.

08-032

ICE AGE RANGE CHANGES, HYBRID ZONES & SPECIATION
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The genetic effects of pleistocene ice ages are approached by deduction from paleoenvironmental information, by induction from the genetic structure of populations and species, and by their combination to infer likely consequences. (1) Recent paleoclimatic information indicate rapid global reversals and changes in ranges of species which would involve elimination with spreading from the edge. Leading edge colonisation during a rapid expansion would be leptokurtic and lead to homozygosity and spatial assortment of genomes. In Europe and North America, ice age contractions were into southern refugia, which would promote genome reorganisation. (2) The present day genetic structure of species shows frequent geographic subdivision, with parapatric genomes, hybrid zones and suture zones. A survey of recent DNA phylogeographic information supports and extends earlier work. (3) The grasshopper *Chorthippus parallelus* is used to illustrate such data and processes. Its range in Europe is divided on DNA sequences into 5 parapatric races, with southern genomes showing greater haplotype diversity - probably due to southern mountain blocks acting as refugia and northern expansion reducing diversity. (4) Comparison with other recent studies shows a concordance of such phylogeographic data over pleistocene time scales. (5) The role that ice age range changes may have played in changing adaptations is explored, including the limits of range, rapid change in new invasions and refugial differentiation in a variety of organisms. (6) The effects of these events in causing divergence and speciation are explored using the grasshopper *Chorthippus* as a paradigm. Repeated contraction and expansion would accumulate genome differences and adaptations, protected from mixing by hybrid zones, and such a composite mode of speciation could apply to many organisms.

08-034

PALEOINTROGRESSION AND REINFORCEMENT IN INSECTS
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The role of reinforcement in increasing and completing reproductive isolation between taxa in secondary contact, by selection against hybrids, has been recently much debated (cf. Butlin, 1985, 1987; Howard, 1993). Crucial points in order to demonstrate reinforcement are: i) to show a lowering of gene flow between interbreeding taxa since they first came into contact; ii) to detect differences in ecological or behavioural prezygotic isolation mechanisms, increasing assortative mating in sympatry.

A number of cases are reviewed in insects, among which the swallowtail *Papilio machaon* and its Sardo-Corsican relative *P. hospiton* (Ungaro *et al.*, in press); the ctenuchid moth *Syntomis* (= *Amata*) *phegea* and its sibling relatives *S. ragazzii* and *S. marjana* (Cianchi *et al.*, in press); the Mediterranean rock-pool hydrenid beetles *Ochthebius quadricollis*, *O. sp. A* and *O. brevicollis steinbuehleri*. (Urbanelli *et al.*, in press). In each case the occurrence of present hybridization and eventual gene exchange is estimated by isozyme markers, as well as the extent of paleointrogression phenomena (*i.e.*, the presence of 'alien' alleles in a species gene pool, owing to past hybridization). Similar cases have been recently found in other animal groups, *e.g.* in amphibians: *Hyla* treefrogs (Verardi *et al.*, in press) and *Hydromantes* plethodontid salamanders (Cimmaruta *et al.*, in press).

08-033

SUTURE ZONES IN INSECTS

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Suture zone theory, announced in 1968, has now had many years of testing and elaboration, with commentary by various investigators.

Three questions to be addressed now are: 1) How extensively are hybrid zones concentrated in suture zones? 2) How long is the duration of clear signals from hybridization following the origin of a suture zone? 3) What is the global geography of the suture-zone phenomenon? There is a close linkage between natural hybridization and suture-zone phenomena, and they will be addressed together.

08-035

BARRIERS TO FERTILIZATION AND REPRODUCTIVE ISOLATION: INSIGHTS FROM A CRICKET HYBRID ZONE

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The ground crickets, *Allonemobius fasciatus* and *A. socius* meet in a mosaic hybrid zone in eastern North America. A characteristic feature of mixed populations is the presence of many "pure" species individuals, a finding which suggests a relatively high degree of genetic isolation between the two taxa. Years of investigation indicate that the isolation is due to a post-insemination barrier to fertilization. I will discuss: factors which may drive the rapid evolution of barriers to fertilization; the isolating potential of such barriers; and our progress in understanding the genetic control of the barrier to fertilization in *Allonemobius*.

08-036

THE ROLES OF CHROMOSOME STRUCTURE, LIFE HISTORY TRAITS AND CLIMATE IN MAINTAINING GENETIC ISOLATION BETWEEN TWO HYBRIDISING TAXA OF THE GRASSHOPPER *CALEDIA CAPTIVA* (ORTHOPTERA: ACRIDIDAE).

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The hybrid zone between the Moreton and Torresian taxa of *Caledia captiva* is extremely narrow and is defined by a series of chromosomal differences that involve all members of the genome ($2n = 22 + XX/XO$). The transition from a Moreton metacentric to a Torresian acrocentric genome occurs over a distance of only 800 metres. Hybrid breakdown among F₂ and backcross generations is 100% and 50% respectively. Similarly, allopatric populations of the Moreton and Torresian taxa show high levels of positive assortative mating ($I = 0.55$). Despite the presence of high levels of postmating isolating factors, high levels of genetic differentiation ($D = 0.23$) between the taxa and the presence of genetic variation for pre-mating isolation, mating within the zone is random with no evidence of disruptive selection favouring positive assortative mating.

An explanation of this apparent anomaly may relate to the instability and movement of the zone during periods of climatic change. Analyses of past biogeographical and current climatic patterns in the area indicate that the zone may have moved during periods of climatic change and its current location is determined by rainfall and temperature seasonality gradients. This is supported by the presence of diagnostic Moreton molecular and biochemical markers in Torresian populations up to 400km to the north of the zones current location.

During the past 5 years the zone has been subjected to drought conditions which predictably should favour the Torresian taxon. An examination of the chromosome structure of the zone has revealed major changes in its composition with evidence of highly significant and asymmetrical movement of Torresian individuals across the zone into predominantly Moreton populations.

The consequences of these rapid and asymmetrical changes to the composition of the zone will be discussed in the context of their impact upon speciation processes in parapatry.

08-037

THE ECOLOGY OF A HYBRID ZONE - CONTACT BETWEEN *HELICONIUS HIMERA* AND *HELICONIUS ERATO* CYRRIA
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Hybrid zones between colour pattern races of *Heliconius* butterflies are maintained by frequency dependent selection acting directly on colour patterns and generally independent of habitat. This study concerns a hybrid zone between two forms which are considered good species, because intermediate forms are rare in the area of contact.

Laboratory experiments have shown that this is due to a strong pattern of assortative mating between the species, without any evidence of hybrid inviability. This talk will concern ecological aspects of the hybrid zone. The cline between the forms is extremely narrow, which suggests that it cannot be explained solely by selection on colour pattern. It is closely correlated with a habitat transition between wet and dry forest, but there is no evidence of divergence in host plant preferences either in the field or laboratory. This suggests that the cline is maintained by competition between the species at the habitat boundary, perhaps for host plant resources. Speciation, in this case, appears to be associated with divergence in habitat and colour pattern.

08-038

HYBRID ZONES AND SPECIATION IN *HELICONIUS* (LEPIDOPTERA: NYMPHALIDAE)

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I give examples of hybrid zones in *Heliconius*, a genus of warningly coloured, mimetic butterfly from South America. There is a continuum from (1) clines between geographic colour pattern forms or races, to (2) examples of hybridization between species that remain virtually distinct, apart from a few hybrids, to (3) parapatric contact zones between species without hybridization. Studies of selection, migration and evolution in this continuum of hybrid/contact zones give important insights into the process of speciation.

08-039

SPECIATION BY HYBRIDIZATION IN STICK INSECTS AND *OTIORHYNCHUS* WEEVILS

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Molecular markers are providing increasing evidence of the occurrence of hybrid species in various insect groups. Speciation by hybridization is a sympatric, allopatric, almost instantaneous event (quantum speciation) giving rise to a new species that combines the genomes of two distinct parental taxa. Individuals of hybrid species, by further hybridizing with bisexual relatives, occasionally give rise to new hybrid forms, often of higher ploidy level (*reticulate evolution*). Insect hybrid species show either clonal (thelytokous parthenogenesis, gynogenesis) or emiclonal (hybridogenesis) reproduction.

A number of cases are examined, among stick insects (genera *Bacillus*, *Clonopsis* and *Leptynia*) and weevils (genus *Otiorynchus*). Short- and long-term evolutionary consequences of speciation by hybridization are discussed.

08-040

TAXON SAMPLING AND HIGHER ORDER MOLECULAR PHYLOGENY: ANALYSIS OF mtDNA SEQUENCES AMONG ORTHOPTEROID INSECTS

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In many of the older insect groups (e.g. the hemimetabolous orders), multiple substitution events in DNA sequences, and particularly mtDNA, have proved a formidable obstacle to phylogenetic reconstruction. Various strategies are available that permit investigators to circumvent problems associated with site saturation. One approach is to use weighting schemes that compensate for the high frequency of certain types of substitutions. Alternatively, an attempt can be made to reduce long branch lengths in phylogenies, where multiple substitutions tend to result in random joining of taxa. I have investigated this second approach using a large, representative sample of orthopteroid mitochondrial DNA sequences, which can be separated into several distinct groups (superfamilies). Numbers of taxa per group were varied and the efficiency of phylogenetic reconstruction assessed by consistency index, bootstrap and PTP analysis. The results confirm that small increases in sampling can have considerable influence on phylogenetic reconstruction. However, the addition of sequences has the advantage of improving the identification of homology in the alignment, and so further augmenting the phylogenetic analysis. This raises the question of whether intensive taxonomic sampling of short DNA sequences may be a more effective strategy of approaching phylogenetic problems than concentrating on longer sequences from smaller taxonomic samples.

08-042

MITOCHONDRIAL DNA VARIABILITY IN THE HONEY BEE (*Apis mellifera* L).

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The mt DNA variability of the honey bee *Apis mellifera* has been investigated by restriction enzyme polymorphism and sequencing. The two types of results are largely congruent and a clear picture emerged from these analyses. Colonies and subspecies are clustered into three major lineages called A, M, and C, geographically structured and distributed as follow:

- an African lineage (A),
- a north mediterranean and caucasian lineage (C)
- a west mediterranean lineage (M)

Theses lineages correspond to a racial and geographical division of the species which strongly confirm Ruttner's hypothesis on morphometrical data, excepted for the North African populations which are assigned to the A branch instead of the M branch. An hypothetical evolutionary scenario has been inferred from these data.

These conclusions are still partial and have been drawn from a limited set of colonies representing a dozen of the 24 subspecies. A much larger survey was necessary to get a better and sounder picture of the differentiation of the species. This is currently being performed with a simple and rapid test, combining PCR of the length polymorphism observed in the COI-COII intergenic region of the mitochondrial genome, followed by a restriction. This test has now been performed on more than 1500 colonies, confirming and extending previous conclusions on the evolution of *Apis mellifera*. Particularly a sub-structuration which had not been observed emerged in the African lineage. In the M lineage, the use of this test gave a good idea of the level of the mitochondrial introgression between lineages due to beekeeping.

08-041

PHYLOGENY AND EVOLUTION OF THE MITOCHONDRIAL CYTOCHROME OXIDASE II GENE IN COLLEMBOLA

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The evolution of the mitochondrial COII gene is analysed in 11 morphological species of Collembola, belonging to four different genera (each one of a different family). The well-known A+T bias, common to all insect mitochondrial genes, is present, although it is lower than that observed in more derived orders, showing a trend to increasing A+T bias in the insect lineage. The number and distribution of variable sites, and the disappearance of the transition/transversion bias in between-genera comparisons suggest that sequences are saturated beyond the genus-level and that relevant corrections to genetic distance estimates should be applied. In addition, high levels of among-site rate variation are observed. The usefulness of the COII gene for reconstructing phylogenetic relationships within Collembola is examined at different taxonomic levels: populations, species, genera and families. Raw sequence divergence is very high at all taxonomic levels, always higher than that observed in other insects. Such high divergence levels may reflect a higher rate of evolution or, more probably, the old age of the groups. The performance of several distance-correction methods, incorporating nucleotide content and TI/TV bias, as well as among-site rate variation, is evaluated. The application of the more sophisticated correction methods reduces the resolution at the deeper nodes, and gives well supported phylogenetic reconstructions only within the genus *Isotomurus*. Distance-based and character-based phylogenetic reconstructions are compared. The lack of resolution at certain deeper nodes may reflect the inability of the COII gene to provide information at these levels or the occurrence of bursts of evolution in the differentiation of collembolan families.

08-043

MtDNA SEQUENCE EVOLUTION IN INSECTS AND THE ADJUSTMENT FOR BASE COMPOSITIONAL BIAS

L. Jermin

ABSTRACT NOT RECEIVED

08-044

MOLECULAR PHYLOGENY OF THE DAMSELFLIES AND DRAGONFLIES AS INFERRED FROM MITOCHONDRIAL 12S rRNA GENES

G. Spicer

ABSTRACT NOT RECEIVED

08-045

NUMEROUS TRANSPOSED COPIES OF mtDNA IN *SITOBION* APHIDS - DISCOVERY AND POTENTIAL APPLICATIONS

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PCR products obtained using primers for mtDNA cytochrome oxidase subunits I and II (mtDNA COI-II) were found to contain multiple haplotypes in three *Sitobion* species. We cloned and sequenced several copies from an individual of each species. Each individual aphid had one mtDNA copy of the sequence, and several other unique sequences shown to be non-mitochondrial by sequencing from purified mtDNA and Southern blotting experiments.

It is most likely that sections of mtDNA have been transposed on many separate occasions into *Sitobion* chromosomes. In these aphids, the phenomenon appears to occur at an almost unprecedented frequency, although it is seen infrequently or not at all in closely-related genera of aphids investigated.

MtDNA copied into the nucleus should be 'fossilized' in as much as it will cease to evolve like mtDNA, and will change at the nuclear pseudogene rate. Thus we believe that *Sitobion* aphids (and other species exhibiting mtDNA transposition) may be important for studying the comparative evolution of mtDNA and pseudogenes.

Our data highlight the need to confirm that putatively mitochondrial sequences obtained in phylogenetic and population genetic investigations are truly mitochondrial.

08-046

DIVERSIFICATION OF THE PHYTOPHAGOUS BEETLE SUPERFAMILY CHRYSOMELOIDEA : COEVOLUTION WRIT LARGE?

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Plant-feeding insects comprise nearly one-half of all insect species, yet nearly three quarters of insect herbivore diversity can be traced to just two origins of plant feeding: in the respective ancestors of the Lepidoptera and of the comparably diverse beetle clade "Phytophaga," which comprises the superfamilies Chrysomeloidea (leaf-, seed- and timber-beetles) and Curculionoidea (weevils). These beetle groups total some 128,000 species, or 10% of all described animal species on earth. The Phytophaga and Lepidoptera both arose in the Mesozoic, but these beetles have conserved much more of the apparent history of their affiliations with major hostgroups, and thus perhaps of their diversification, than have butterflies and moths. While most of these beetles are today affiliated with flowering plants, fossils document their first appearance in the Jurassic, before flowering plants arose. Indeed, the current affiliations of the morphologically most primitive subfamilies to which these fossils belong are with conifers and cycads (i.e., plants found in the same fossil beds), while groups of intermediate and advanced position occupy similarly positioned plants. In conjunction with the newest, DNA-based estimate of higher plant phylogeny and evidence from the substantial fossil record, analyses of over 100 complete 18S gene sequences and of morphological characters for these beetle subfamilies indicate that the current hostplant affiliations of these insects strongly reflects their ages of origin. Moreover, colonizations of newly-diversifying hostplant producers by these consumers are associated with much enhanced diversity, suggesting that innovations in lifestyle may spur herbivore radiation, with implications for assembly and diversity of insect/plant communities. It would also seem that such affiliations as are conserved over geological time and global space may, perhaps, be less reasonably ascribed to stabilizing selection than to paucity of genetic variation, bearing implications for extinction of herbivore groups whose hosts disappear.

08-047

INDUCED RESISTANCE IN PLANTS AGAINST GALL-FORMING INSECTS: ACTIVE DEFENSE OR LACK OF RECOGNITION? Stig Larsson, Carolyn Glynn & Solveig Höglund
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Gall-forming insects induce their host plants to supply them with shelter and food. A common feature of these organisms is that they manipulate their host by use of an intricate chemical signal system. If the signals fail, the gall maker is unable to initiate and maintain the necessary contact with the host plant; the gall is unsuccessful and the gall maker dies.

This study highlights the elements of close association between a willow, *Salix viminalis*, and the leaf-rolling cecidomyiid *Dasineura marginemtorquens*. Certain willow individuals have very few galls, due to high larval mortality, while plants growing beside suffer heavy gall midge infestation. The resistance is genetically based and is not of a constitutive nature. We suggest two alternative hypotheses as an explanation for the resistance in this system. First, analogous to what has been observed in many plant/microorganism interactions, the attacking larva may induce a signal that through a number of intermediate steps trigger the expression of defense genes. The result would be the active defense and a killed larva. Second, and specific to the situation in gall-forming organisms, the resistant genotype may not respond appropriately, from the insects point of view, and therefore the gall will not be induced. The result in this case would be that the larva starve to death.

The strong intraspecific variation in resistance that we have documented is unusual among herbivorous insects, and resembles more the situation for microorganisms. We suggest that the galling habit is responsible for this; the gall initiation is a very intricate process involving the transmission of specific chemical compounds from a rather sessile larva to the plant with opportunities for the plant to respond accordingly.

08-048

EVOLUTIONARY ECOLOGY OF ANT-PLANT SYMBIOSES:
INCLUDING ANTS IN THE ANALYSIS

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Studies of the evolutionary ecology of symbiotic ant-plant mutualisms have usually been tacitly viewed from the plant's perspective, as if the biology of the ant partners could be reduced to the services (protection, feeding of the plant, etc.) provided to the plant. However, selection pressures acting on the ants, especially at the evolutionary outset of these symbioses, are complex and do not always act to reinforce mutualism. Recognition that the evolutionary interests of ants and plants in these symbioses are not entirely congruent opens up a number of new avenues for research, in which parasitism and evolutionary conflict appear as themes alongside mutualism.

These new lines of research concern (1) life history evolution of plant-ants in relation to coevolutionary interactions with plants; (2) how plants control investment in maintaining ants and the importance of this control for stability of the mutualism; (3) the evolution of parasitic strategies in these globally mutualistic interactions; and (4) conflict and cooperation in ant societies in relation to ant-plant coevolution.

08-050

EVIDENCE OF EVOLUTION OF FOOD-PLANT ASSOCIATIONS
SHOWN BY FAMILIES OF PHYTOPHAGOUS BRITISH INSECTS
AND MITES

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Data from food-plant records of British phytophagous insects and mites are used in a correspondence bi-plot analysis to demonstrate the interrelationships of all 6935 species of insects/ mites and about 2000 plants. These were arranged in a matrix of 186 insect/mite families and 117 plant families. The complex figure plotted from the first two axes of variation shows all insect and plant families together, and is used in a pattern analysis in which taxonomic and evolutionary relationships are shown to be important.

Insect and plant families in the analysis were ranked as 'primitive', intermediate or advanced. Sporne's index of advancement was used for dicots. The results indicate that many of the more advanced insect families are associated with more advanced plant families and visa versa. Advanced plant families are geologically younger, herbaceous and have more diverse secondary compounds, while primitive/more generalized plant families tend to be geologically older, woody and less diverse. Most of the phytophagous orders of insects/mites show some evidence of phylogenetic sequencing of family associations relating to the broad phylogenetic levels of plant groups. Elements of this same sequencing, summarized to the classes of plants, can be detected within insect families, although this may be obscured by switches to unrelated food-plants.

08-049

PATTERN AND PROCESS IN THE EVOLUTION OF INSECT-HOST
PLANT ASSOCIATIONS

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Phylogenetic studies are increasing our understanding of the evolution of associations between plants and insect herbivores. Coevolution where reciprocal selection between interacting insects and plants is supposed to induce chemical diversification and resistance in plants and food specialization in insects appears to be less common than sequential evolution, i.e. the shift of insect herbivores onto pre-existing plant species.

Extreme host specificity is common in phytophagous insects. Hypotheses that assume that food specialists have selective advantages over generalists do not seem to provide a general explanation for the ubiquity of specialists. Specialists are probably committed to remain so, because they have little evolutionary opportunity to reverse the process due to genetically determined constraints on the evolution of their physiology or nervous system. The same constraints might result in phylogenetic conservatism, i.e. the frequent association of related insect herbivores with related plants. Current phylogenetic evidence, however, indicates that there is no intrinsic direction to the evolution of specialization.

Historical aspects of insect-host plant associations will be illustrated with the small ermine moth genus *Yponomeuta* (Lepidoptera, Yponomeutidae). Small ermine moths show an ancestral host association with the family Celastraceae. The genus seems to be committed to specialization per se rather than to a particular group of plants. Whatever host shift they have made in their evolutionary past (onto Rosaceae, Crassulaceae, and Salicaceae), they remain monophagous.

A scenario will be discussed in which sensitivity to dulcitol might have facilitated the shift from Celastraceae to the taxonomically unrelated and phytochemically dissimilar Rosaceae. Dulcitol is the major sugar alcohol in Celastraceae where it acts as a phagostimulant for larvae and possibly as an oviposition stimulant in adult females. It also occurs in low concentrations in species of the genus *Prunus* (Rosaceae), the very host plants of a number of *Yponomeuta* species.

08-051

GENE TREES, CHROMOSOME TREES, SPECIES TREES:
MOLECULAR PHYLOGENIES IN THE *ANOPHELES GAMBIAE*
COMPLEX.

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The increasing number of studies using DNA sequences has produced a large number of papers where phylogenetic considerations of the relation of species are drawn based on single gene data. In this communication I would like to highlight the danger of confining molecular phylogenetic analysis to a single DNA region especially in recently diverged taxa. I will use the molecular studies carried out on the *Anopheles gambiae* complex as a case study to discuss this problem, taking advantage of the unusually detail knowledge of the biology of the group and of the chromosome location of the gene analyzed. Such information were essential in comprehending the conflict between different gene phylogenies.

The *Anopheles gambiae* complex includes the most efficient vectors of malaria in the world. The complex is confined to sub-Saharan Africa and consists of six closely-related sibling species. Until recently, the only information on the phylogenetic relationship among these species had been derived from chromosomal inversions. The use of these chromosomal variants relies on the assumption that existing inversions are monophyletic. Virtually all phylogenetic trees derived from molecular data have confirmed this assumption in other taxa. A recent exception was found in the *Anopheles gambiae* complex, where DNA sequence data from the mitochondria (mtDNA) and the nuclear rDNA intergenic spacer produced phylogenies in disagreement with the inversion tree. A simple explanation for this conflict could be that the inversions at study are not monophyletic. Because inversions protect genes within breakpoints from recombination, if inversions are monophyletic, then DNA sequences within inversions should reflect the evolutionary histories of the inversions. To test the monophyly of 3 important inversions in this group (X^{a9} , $2L^{a/+}$, and $3R^{a/+}$), DNA sequences within and on the breakpoints were analyzed from the six species included in the complex: *A. gambiae*, *A. arabiensis*, *A. merus*, *A. melas*, *A. quadriannulatus*, and *A. bwambae*.

08-052

PRELIMINARY STUDIES OF ECOLOGICAL AND GENETIC ISOLATION OF *ANOPHELES BWAMBAE* OF THE GAMBIAE COMPLEX (DIPTERA: CULICIDAE)

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Studies were conducted in the vicinity of the geothermal springs in Bwamba County, Uganda to assess the degree of ecological and genetic isolation between three species of the Gambiae Complex: *An. bwambae*, a species known only from the geothermal springs environment, *An. gambiae* and *An. arabiensis*. The results of DNA identification and oviposition tests indicate that the larvae of *An. bwambae* and *An. gambiae* may occupy the same habitats in drainage water derived from the springs. Variability of adult and larval morphology and isozyme polymorphisms at the ODH and SOD loci show evidence of incomplete divergence between these two species, while the use of DNA primers for species identification indicate that they are well segregated. *Anopheles bwambae* and *An. arabiensis* appear to be essentially parapatric.

08-053

A MOLECULAR PHYLOGENY FOR THE *DROSOPHILA MELANOGASTER* SUBGROUP.

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Drosophila melanogaster belongs to a closely-related group of eight species collectively known as the *melanogaster* subgroup; all are native to sub-Saharan Africa and Islands off the east coast Africa. The phylogenetic relationships of most species in this subgroup have been well-documented; however, the three most closely related species, *D. simulans*, *D. sechellia*, and *D. mauritiana*, have remained problematic from a phylogenetic standpoint as no data set has unambiguously resolved them. We present new DNA sequence data on the *nullo* gene and combine it with all available DNA sequence data; the total data set are 11 genes and the ITS of rDNA. A methodological problem had to be overcome as several of the genes had considerable information on intra-specific polymorphisms. We adopted the strategy of eliminating all polymorphic sites as they represent either ancient polymorphisms which could lead to gene tree/species tree problems, or are autapomorphies and thus contain no phylogenetic information. We have also re-analyzed our previous DNA-DNA hybridization data with a bootstrap procedure. The combined sequence data set and the DNA-DNA hybridization data strongly support the sister status of the two island species, *D. sechellia* and *D. mauritiana*. This at least partially resolves what had been a paradox of parallel evolution in these two species.

08-054

PHYLOGENY OF *Dolichopoda* CAVE CRICKET SPECIES BASED ON SATELLITE DNA SEQUENCES

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Dolichopoda cave crickets are currently the object of extensive research in our laboratory with the aim to investigate evolutionary relationships among populations and species by means of several markers with different informational value in respect of phylogeny and adaptation. In recent years we are investigating tandemly repeated satellite DNA a class of non-coding DNA supposed to be fast evolving and selectively neutral. We have isolated and characterized three families of satellite DNA, two of which are species specifically amplified only in one species of *Dolichopoda* while the third one is widely distributed throughout the entire genus. One typical feature of highly repeated DNA families, common to all satellite DNAs so far analysed is its high intraspecific sequence homogeneity contrasting high interspecific diversity. Sequence data of each satellite DNA family from different *Dolichopoda* populations were used to generate trees: as expected, sequences derived from conspecific populations cannot be discriminated. However, sequence data from the satellite sequences spread over the whole *Dolichopoda* genus generate trees showing homogeneous clusters of sequences, which are consistent with the specific rank of populations: species can clearly be discriminated while conspecific populations are not. The phylogenetic relationships inferred from this family of sequences are in agreement with those derived from other molecular markers such as allozyme polymorphisms, DNA-DNA hybridization data and mitochondrial RFLPs; this suggests a role for satellite DNA as a marker for cladogenetic events.

08-055

ENZYMATIC ANALYSIS OF THE AZOREAN, PORTUGUESE MAINLAND AND CANADIAN POPULATIONS OF *MYTHIMNA UNIPUNCTA* (HAWORTH) (LEP., NOCTUIDAE)

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Polyacrylamide gel electrophoresis was used to evaluate the genetic population structure of the armyworm, *Mythimna unipuncta* (Haworth) (Lepidoptera: Noctuidae), an agricultural pest in the Azores archipelago. Electrophoretic analysis of four enzyme systems (aldehyde oxidase, esterase, phosphoglucose mutase, phosphoglucose isomerase) was performed on six populations from azorean islands (Santa Maria, São Miguel, Terceira, Pico, Faial, Flores), which were compared with those for the Portugal mainland (Europe) and Canada (North America). The band patterns were described, compared and interpreted from both genetic and evolutionary point of views.

08-057

USE OF RANDOM AMPLIFIED POLYMORPHIC DNA POLYMERASE CHAIN REACTION (RAPD-PCR) TO DETECT DNA POLYMORPHISMS IN *Dacus oleae* (DIPTERA: TEPHRITIDAE).

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The polymerase chain reaction (PCR) was used to generate random amplified polymorphic DNA (RAPD) from olive flies DNA samples in order to assess the degree of polymorphism within the genus and to investigate if this approach was suitable for genetic studies of this species.

Genomic DNA was extracted from 20 individual *Dacus oleae* flies from two geographic populations and amplified in PCR reactions using single primers of arbitrary sequence. Sixteen different oligonucleotide primers (C1, C2, C3, C4, C5, C6, C7, C8, C10, C11, C14, C15, C16, C18, C19, C20, Operon Technologies) yielded clear and reproducible bands corresponding to amplified products and separable by agarose gel electrophoresis. A total of 215 bands were obtained. Of these, 155 bands were variables, and 26 were present in only one of the populations. The intra and interpopulational mean values of Jaccard (0.6727 and 0.6682, respectively) were not statistically different. The results are discussed in relation to the suitability of the technique in population genetic studies of agricultural pests.

08-056

USE OF MOLECULAR MARKERS FOR RACE DIFFERENTIATION OF THE GYPSY MOTH *LYMANTRIA DISPAR* L.

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The first observation of flying gypsy moth females - so far known as being characteristic only for Asian gypsy moth populations - in Southwest Germany in 1993 supported the conclusion that the Asian race of the gypsy moth has become established in Germany. Differentiation by "traditional" morphological characteristics is not successful in clearly distinguishing both the European and Asian race of the gypsy moth. The use of various molecular markers based on the polymerase chain reaction (PCR) resulted in characteristic DNA fingerprints which could be used for positive identification of the different races in Southwest Germany. Additional studies indicated that gypsy moth females from different German populations are indeed capable of sustained flight, but that this ability is strongly correlated to their geographic origin. The interpretation of this behavior is discussed on the basis of the molecular genetic approach.

08-058

RANDOM AMPLIFIED POLYMORPHIC DNA OF SCREWORM FLY POPULATIONS (DIPTERA: CALLIPHORIDAE) FROM BRAZIL.

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The screwworm fly, *Cochliomyia hominivorax* (Coquerel, 1859) one of the most important pests causing invasive myiasis in warm-blooded vertebrates in Brazil. Several studies at molecular level have been conducted to analyze the genetic variation in natural populations of this species. In this work we have attempted to characterize the polymorphism of *C. hominivorax* using genomic markers.

The PCR based technique involving the random amplification of polymorphic DNA (RAPD) produces diagnostic DNA fragments, which can be used to characterize and differentiate insect populations. This technique was optimized and used to study the genomic variability among four *C. hominivorax* populations in São Paulo State (Adamantina, Amparo, Botucatu and Valinhos). Genomic DNA of *C. hominivorax* was tested using eleven different DNA concentrations for amplification conditions optimization. Reproducible bands were amplified until 100 µg of DNA.

RAPD fingerprints presented high variation for ten primers used (Operon Technologies, Alameda, CA), giving 298 polymorphic bands out of 389, ranging from 520 to 3000 bp in size. The average number of polymorphic bands was 7.5 per primer. The OPB-02, OPB-06 and OPB-09 primers were able to detect a high number of polymorphic bands in all *C. hominivorax* populations.

An estimate of within and between population dissimilarity based on the dissimilarity index (*d*) was calculated and can improve discrimination in *C. hominivorax* populations. The within population dissimilarity values ranged from 9.5% (Valinhos) to 11.7% (Botucatu), and the between population dissimilarity values ranged from 6.9% (Amparo v/s Valinhos) to 10.6% (Adamantina v/s Botucatu).

This genomic polymorphism detected with RAPD-PCR in populations of *C. hominivorax* has indicated that this species has a high level of variation in tropical habitats, with differentiation of populations, as was suggested by the mtDNA analysis.

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08-059

KARYOTYPIC STRUCTURE TRANSFORMATION OF
SIBERIAN MALARIA MOSQUITOES (DIPTERA:
CULICIDAE) AS A RESULT OF GLOBAL WARMING

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Historically, ecological diversification and occupation of new niches during the adaptive radiation of Palearctic mosquito species depended on 2 principal selection strategies. Apparently, K-strategy was the initial one in the group of *Anopheles maculipennis* sibling species, which includes *An. messeae*. Expansion of *An. messeae* to the northeast of the Palearctic was associated with changes in selection strategy and origination of "northern" chromosomal inversions. Mosquitoes with combinations of such inversions are r-strategists. Nowadays there is a strong trend towards frequencies increasing of "southern" karyotypes in the populations of the West Siberia as the result of global warming. Exclusion of r-strategists gives rise to a new system of biotic connections in the mosquito breeding places.

08-061

METAPOPULATION STRUCTURE IN *APHIS GOSSYPHII* IN
GREENHOUSES: EVIDENCE FROM MOLECULAR MARKERS

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Random amplified polymorphic DNA markers and allele variation at microsatellite loci were used to study the genetic diversity of populations of aphids, *Aphis gossypii*. Aphid clones were collected in two greenhouses (50 km apart) on several host plants and outdoor surrounding plants, two times during the plant infestation process (founding and culminating) and over three successive years. Beside the genetic polymorphism detected within each sample, factorial analyses revealed a statistically significant variation in the population genetic structures in relation to year, site, host plant and time of sampling. The results provide evidence that local populations of aphids are not persisting in each site from year to year but experience frequent extinction recolonization processes. Moreover, there was an increase of genetic polymorphism among aphid clones sampled in one greenhouse during the plant infestation process. The most likely explanation for this variability is to consider that dispersion of aphids is occurring among local populations. The results suggest that the long time persistence of *Aphis gossypii* populations can be explained by the metapopulation model.

08-060

GENE FLOW AND THE GEOGRAPHIC STRUCTURE OF
NATURAL POPULATIONS OF *CERATITIS CAPITATA*.

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Five populations from African region (Kenya, Reunion, Morocco, Canary, Madeira), nine Mediterranean (Spagna, Sardinia, Procida, North Italy, Libya, Israel, Creta, Chios, Greece) and three from the New World (Hawaii, Guatemala, Australia) for a total of 82 samples, were examined by electrophoresis for genetic variability at 26 enzyme loci (MLEE approach).

Wright's F_{ST} , Slatkin's estimates, together with tree representations have been used to compare the African populations with the derived ones. Parameters using gene frequencies (F_{ST} , D , N_m) indicate the presence of substantial geographic heterogeneity correlated with the dispersion of medfly from its source area (Sub-saharian Africa) to the periphery. Significant gene flow estimates between African and the derived populations support the hypothesis of a recent colonization of *C. capitata*.

These results are significantly correlated with those derived from DNA data both with RAPDs (Baruffi et al., 1995, *Heredity*, 74: 425-437) and scDNA polymorphism (Gomulski et al., accompanying poster).

08-062

Study of the biology and distribution
of the Russian wheat aphid. *Diuraphis*
noxia(Mordvilko)(Hom-Aphididae) in
Tehran province.

Gh. Reza. Rassoulia and L. Doulati.

Our investigations on the biology of Russian wheat
aphid in green house and field conditions resulted:

- 1) The average time to complete the nymph stage was 7.5 days.
- 2) The average of pre reproductive, reproductive, postreproductive periods and total longevity were 1,42.88, 11.63 and 63 days respectively.
- 3) The average number of nymphs produced by each parthenogenetic female was 72.12.
- 4) At the field condition, the average time required to complete nymphal stage was, 19.71. the average for pre reproductive, reproductive, post reproductive periods and total longevity was 2.43, 49, 43, 87.43 days respectively. average nymph for each aphid was 76.80. our studies indicated, Russian wheat aphid over winters on some grasses and cereal hosts as parthenogenetic female. The investigations showed that, *D. noxia* was distributed at many areas of Tehran province as Varamin, Damavand Talegan, Shahriar and Ghazin.

08-063

DISCRIMINATION OF DIFFERENT CLONES OF *APHIS GOSSYPHII* GLOVER (HOMOPTERA: APHIDIDAE) BY RAPD-PCR

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Total DNA was extracted and purified from 5 adult virginoparous females of *Aphis gossypii* clones cultured in the laboratory. DNA was amplified in the 20 μ L PCR buffer with 0.5 μ L of template DNA (1 ng / μ L), 1 μ L of primer (20 pM / μ L) and 1 μ L of Taq DNA polymerase. Five aphid clones differing in life-cycle and six clones differing in insecticide resistance were used for experiments. These clones are tested by RAPD with each of 92 random primers; Operon's ten- or Wako's twelve-nucleotide oligomer. Amplification reactions were carried out for 45 cycles of 1 min at 92°C, 1 min at 35°C and 2 min at 72°C. Several fragments of DNA were amplified in each clone with each primer. Banding patterns generated from some primers appeared to distinguish different clones having different biological traits. These primers shall be tested for other clones to assure the general applicability.

08-065

INTRASPECIFIC VARIABILITY IN HOST PLANT ADAPTATION OF THE GREENBUG, *SCHIZAPHIS GRAMINUM* ROND. (HOMOPTERA: APHIDIDAE)

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An analysis of greenbug clones, sampled during 1993-1995 from sorghum fields at the Cuban'Exp. Sta. of VIR (Krasnodarskii Krai), revealed a wide variability in host plant exploitation by the pest. It has been possible to classify the aphid into at least 6 phenotypes which differ in their ability to destroy sorghum lines with specific major genes for resistance. We tested the Sarvasi cultivar, used widely in breeding programs, the donors of resistance employed in the USA (KS-30, Capbam, PI 264453) and the new resistance donors (k-924, k-1362, k-9436). Sarvasi and the lines with genes identified in the USA and which Russian greenbug populations have never met, are susceptible. However, we have found some clones avirulent to Sarvasi, Capbam and PI 264453 for 3 years. Most of the clones were avirulent to the gene in k-9436 and two clones were virulent to the genes in k-9436 and k-1362 in 1993 only. The sample k-924 is highly resistant to all the clones. The lines with noneffective genes have a low level of resistance to all virulent clones. This may be a result of "residual" effect of oligogenes or an effect of minor genes. The population diversity is attained by mutations, recombinations (the main greenbug gene pool in Russia is holocyclic) and assimilation of immigrants. This diversity is a base for aphid adaptation to new resistant sources. Further studies are in progress to develop a sorghum differential set.

08-064

ROUTES TO SOCIALITY IN *PEMPHIGUS* (HEMIPTERA: PEMPHIGIDAE) - MORPHOMETRIC CLUES.J.A. Whitfield, N. MacLeod¹

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British species of the gall forming aphid genus *Pemphigus* display a wide diversity of life history and behavioural traits. In particular, *Pemphigus spyrothecae* Pass. is unique in its loss of host alternation and its production of dimorphic first instars in the third gall generation, including an altruistic defensive morph.

Morphometric analysis of *Pemphigus* first instars shows that *P. spyrothecae* "soldiers" are extremely similar to the first instars of the second gall generation found in all examined gall-forming *Pemphigus*, while it is thought that the "non-soldiers" are equivalent to the monomorphic secondary host generations of host alternating species. A major evolutionary innovation, then, in *P. spyrothecae* is the production by apterac of second gall generation "soldier" type first instars in the third gall generation.

Although the gall generations of several *Pemphigus* species show attacking behaviour, *P. spyrothecae* shows additional morphological adaptations that seem to suit it to a soldier role. These include relatively thicker hind femurs and broader, more hooked claws on the hind legs - the limbs most important in attacking behaviour - than host-alternating species.

This combination of behavioural, life cycle and morphometric data points to the sequence of evolutionary events in *Pemphigus* being the evolution of attacking behaviour in a number of species, followed in *P. spyrothecae* by the loss of host alternation, the production of soldiers in the third gall generation and morphological specialisation of soldier type first instars.

08-066

MORPHOMETRIC STUDY OF *DROSOPHILA SERIDO* POPULATIONS FROM CENTRAL AND SOUTHERN REGIONS OF BRAZIL.S. G. Monteiro, F. M. Sene¹

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Drosophila serido is an endemic species in South America. Analysis of various markers has shown that this species is excellent material for studies on evolution, due its considerable diversity. Aedeagus morphology is the main feature used to separate species and to identify populations that are in a differentiation process. Aedeagus morphology was analyzed in 18 populations of *D. serido*, from central and southern regions of Brazil. Multivariate analyses of the data confirmed the existence of two distinct groups of aedeagi, named B and D. Measurements of the two superior curvatures of the aedeagus best discriminate the two types. There was a relationship between types of aedeagus and species of cactus: type B is associated with *Philosocereus machrisii*, found in high altitude cerrado, while type D is associated with *Cereus hildemannianus*, normally found along river valleys. Analysis of populations of each type has show that populations found on hills, classified as type B, are heterogeneous while those found along the rivers (type D) are more homogeneous. This implies geographical isolation of type B populations and considerable gene flow between type D populations, which would affect differentiation and consequently speciation.

08-067

MORPHOLOGICAL AND GENE-ENZYME VARIABILITY OF SYMPATRIC POPULATIONS OF THE SPECIES *Aedes caspius* (Pallas, 1771) and *Aedes dorsalis* (Meigen, 1830) (Diptera: Culicidae)

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Genetic variability of sympatric populations of the species *Aedes caspius* and *Aedes dorsalis*, from the soil lake Rusanda (Yugoslavia), performed by analyzing morphometric characters and gene-enzyme variability was studied. Morphological characters have been analyzed on the IV instar larvae. Statistically significant differences were observed between the mean values of the number of pecten teeth and the siphon-length index (but not in number of teeth in the siphon tuft, siphon index, area and circumference of the siphon).

By the PAG electrophoresis eight enzyme controlled by nine loci: *Gpd-2*, *Gpi*, *Me-2*, *Idh-2*, *Hbd*, *Odh*, *Est-1*, *Est-5* and *Pgm* were analysed. Genetic variability of *Ae. caspius* showed: 33.3% polymorphic loci, 3.7 mean number of alleles per locus, and 30.5% expected mean heterozygosity and that of *Ae. dorsalis* 33.3% polymorphic loci, 3 mean number of alleles per locus, and 30.7% expected mean heterozygosity. The genetic distance between these two species was 0.557.

08-069

THE USE OF MORPHOMETRICS OF MALE GENITALIA AND FEMALE WING VENATION IN THE IDENTIFICATION OF *CULEX PIPIENS* L. COMPLEX IN IRAQ

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The two mosquitoes, *Culex quinquefasciatus* say (Baghdad strain) and *Culex pipiens molestus* Forskal (Mosul strain) could be differentiated based on the characters of male genitalia and female wing venation. The D/V ratios for *Cx. quinquefasciatus* and *Cx. p. molestus* were 0.337 and 1.434 and the DV/D ratios were 1.054 and -1.137 respectively on average. The R-cell and M-cell ratios of the right wing venation of *Cx. quinquefasciatus* and *Cx. p. molestus* were 303.08 and 347.66 (R-cell) and 185.02 and 170.17 (M-cell) respectively. The mean values of M- and R-cell ratios were significantly different between the two mosquito species with a marginal error.

08-068

EVIDENCE FOR PHENOTYPIC SEXUAL SELECTION OF A MALE GENITALIC CHARACTER IN THE SEEDBUG *LYGAEUS SIMULANS* (HETEROPTERA: LYGAEIDAE)

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The female choice theory of genitalia (Eberhard 1985) predicts the existence of phenotypic sexual selection of male genitalia mediated by female choice. To evaluate this, a selection experiment was conducted with 48 laboratory bred virgin males of *Lygaeus simulans* Deckert. Each male had the opportunity to copulate with three virgin females. Mating success was judged by occurrence of sperm in the female spermatheca 24h after copulation.

Only 60% of the copulations were successful. A Lande-Arnold analysis (multiple regression of mating success against genitalic and non-genitalic male characters) indicates direct stabilising selection on a morphological genitalic character of males (length of processus gonopori).

08-070

REDUCED VARIATION IN *DROSOPHILA SIMULANS* MITOCHONDRIAL DNA

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Two recent studies of DNA sequence polymorphism in *Drosophila simulans* mitochondrial genes found evidence for reduced variation, indicating a recent hitchhiking event accompanying natural selection. To test whether the lack of variation in *D. simulans* mtDNA is associated with a current *Wolbachia* infection we sequenced three regions of the mitochondrial genome in 36 uninfected and four infected wild-caught isofemale lines from a single population in Lantana, Florida. Mitochondrial variability is significantly reduced in both infected and the pooled sample of infected and uninfected flies compared to a noncoding region of the nuclear-encoded gene, *Adh*^r. The loss of mitochondrial variation must have occurred prior to the current *Wolbachia* infection, possibly by genetic hitchhiking accompanying selection at linked sites. Alternative scenarios to explain this unexpected result will be discussed.

08-071

WOLBACHIA INFECTION AND CYTOPLASMIC INCOMPATIBILITY IN DROSOPHILA SPECIES

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Wolbachia, a maternally inherited microorganism, was first described in the *Culex pipiens* complex and it has since been associated with cytoplasmic incompatibility, thelytoky and feminization phenomena in a variety of arthropods. Cytoplasmic incompatibility in insects results in either embryo mortality or production of all-male progeny from a cross between an infected male and an uninfected female and it has been reported in diverse insect taxa.

In the present study, forty-one stocks from thirty different *Drosophila* species were surveyed for *Wolbachia* infection using PCR amplification of *dnaA* and 16S rDNA sequences. *D. sechellia* and two strains of *D. auraria* were found to be infected and were tested for the expression of cytoplasmic incompatibility, along with *D. ananassae* and six strains of *D. melanogaster*, which are already known to be infected. *D. ananassae* and *D. melanogaster* show levels of incompatibility up to 25%, while *D. auraria* and *D. sechellia* exhibit levels of egg mortality higher than 60%. A dot-blot assay using the *dnaA* sequence as probe was developed to assess the infection levels in single flies. The stocks examined were classified into two groups, depending on the levels of infection relative to the degree of cytoplasmic incompatibility exhibited. One group, containing *D. simulans* Hawaii, *D. sechellia*, and *D. auraria*, exhibits high levels of cytoplasmic incompatibility relative to levels of infection; all the other species and *D. simulans* Riverside belong to the other group, which exhibits significantly lower levels for cytoplasmic incompatibility relative to infection levels. A positive correlation between bacterial density and cytoplasmic incompatibility was observed within each of the two groups. These data show that, in addition to bacterial density, bacterial and/or host factors affect the expression of cytoplasmic incompatibility.

08-073

THE HYBRID ZONE BETWEEN TWO SUBSPECIES OF CORN ROOTWORM BEETLES, *DIABROTICA VIRGIFERA VIRGIFERA* AND *D.V. ZEA*, IS INFLUENCED BY A *WOLBACHIA* INFECTION.

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We report that *Wolbachia* bacteria are the cause of the unidirectional reproductive barrier observed between the subspecies of the chrysomelid beetles *Diabrotica v. virgifera* and *D. v. zea*, two major pests of maize commonly known as the Western and Mexican corn rootworms. Previous work on these Chrysomelid beetles has revealed that few eggs hatch from the cross of *D. v. virgifera* males with *D. v. zea* females. Using PCR primers specific to the 16S ribosomal RNA of *Wolbachia*, populations of *D. v. virgifera* were sampled throughout the range of this subspecies and were consistently found to be infected with *Wolbachia*, but *D. v. zea* populations tested were not infected. Treatment of a *D. v. virgifera* stock with tetracycline to remove the *Wolbachia* removed the mating incompatibility with *D. v. zea* females. We infer that the present population of *D. v. virgifera* acquired the *Wolbachia* infection in Mexico, its putative site of origin shared with *D. v. zea*, prior to spreading to its present range, and that *D. v. zea* may be the parental population. The subspecies status of *D. v. virgifera* and *D. v. zea* is largely attributed not to intrinsic genetic differences but on the basis of infection with an endosymbiont. Given the relatively common presence of *Wolbachia* in insects it is likely that other insect hybrid zones may be in part determined by an infection with this bacterium.

08-072

SPATIAL AND TEMPORAL DYNAMICS OF INFECTION WITH *WOLBACHIA*, CYTOPLASMIC INCOMPATIBILITY MICROORGANISMS, IN NATURAL POPULATIONS OF SMALL BROWN PLANTHOPPER, *LAODELPHAX STRIATELLUS* (HOMOPTERA: DELPHACIDAE), IN CENTRAL JAPAN

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Cytoplasmic incompatibility (CI) is caused in various insects by intracellular infection with rickettsia-like microorganisms of the genus *Wolbachia*. The theoretical viewpoints expect *Wolbachia* infection to spread in host population due to unidirectional CI, although information on spatial and temporal dynamics of *Wolbachia* infection in natural insect population is limited except for *Drosophila simulans*. In Japan, *Laodelphax striatellus* shows unidirectional CI between northeastern and southwestern populations. In 1994, nine natural populations of *L. striatellus* collected from central Japan were analysed for *Wolbachia* infection by the polymerase chain reaction (PCR) using primers specific to *Wolbachia* 16S ribosomal DNA. The infection rates appeared to show clinal decrease from southeast to northeast. Comparison with a previous study suggested that in some populations of *L. striatellus* the infection property had changed from the uninfected to the infected. Based on such geographic and temporal pattern, it was concluded that *Wolbachia* infection in *L. striatellus* populations have spread northeasterly during 1982-1994. More detailed geographic pattern of *Wolbachia* infection polymorphism will be discussed and temporal dynamics of infection will be inferred based on the results of PCR assay for samples collected in 1995.

08-074

WOLBACHIA INFECTIONS IN THE FLOUR BEETLE *TRIBOLIUM CONFUSUM*: EVIDENCE FOR A SINGLE AND COMMON CYTOTYPE AMONG STRAINS

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The flour beetle *Tribolium confusum* is one of many insect species known to harbor a rickettsial symbiont (*Wolbachia*) that causes cytoplasmic incompatibility in its hosts. Despite its maternal pattern of transmission, parasite-host phylogenies indicate *Wolbachia* has been transferred horizontally, even across distantly related host species. In addition, some insects are also known to harbor infection with two different strains of *Wolbachia*. This is of evolutionary interest as it raises questions of how these double infections can be acquired and maintained.

We surveyed 12 geographical strains of *T. confusum* for infection using *Wolbachia*-specific PCR primers. We also tested 8 infected strains for differences in their compatibility types by single-pair matings between infected individuals. The results show that the strains tested are infected with a common incompatibility type. Finally, we surveyed all infected strains for double *Wolbachia* infections by using specific PCR primers and diagnostic PCRd analysis. The molecular data show that all infected strains harbor a single and common cytotype, which correlates with the results of mating tests.

08-075

GENETIC VARIABILITY OF THE RESISTANCE TO HIGH TEMPERATURES AND ANTIBIOTICS IN A HOST-SYMBIOTE SYSTEM

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Several *Trichogramma* species (Hymenoptera: Trichogrammatidae) live in symbiosis with intracellular microorganisms belonging to the genus *Wolbachia*, which induce a thelytokous parthenogenetic reproduction. *Trichogramma cordubensis* Vargas & Cabello, a species living in southern Europe and northern Africa, is entirely infested in the wild. However, in summer, a natural thermotherapy can restore a bisexual reproduction. We verified the existence of a genetic variability of the resistance to high temperature (30°C) and to minocycline (10% in solution) in a host-symbiote system (*T. cordubensis*-*W. trichogrammae* Louis & Pintureau) from Azores. Treatments (rearing at 30°C, imagos supplied with diluted honey + minocycline) were applied for 3 generations (G1-G3). *Trichogramma* pairs (20 at 30°C and 28 with minocycline) were constituted at the G3 and supplied with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as hosts. Offspring of the obtained lines was isolated, and 4 to 18 pairs were constituted in each line and supplied with hosts. Sex ratios (male and intersex* percentages) were calculated in offspring of these pairs. As usually done to estimate genetic variability, we compared the within- and between-line variances (sex ratios were 2 arc sin $\sqrt{}$ transformed) by one-way analyses of variance (anova). Results of the analyses:

Anova	30°C			Minocycline		
	% males	% intersexes	% males + intersexes	% males	% intersexes	% males + intersexes
F	3,829	1,512	4,087	3,286	3,144	3,272
p	0,0001	0,0904	0,0001	0,0001	0,0001	0,0001

Thelytoky is more or less persistent, i.e. "Individuals" (1 *Trichogramma* + n *Wolbachia*) show a more or less important resistance to high temperatures and antibiotics. A part of this variability has a genetic origin but we cannot know if responsible genes belong to the host genome, the symbiote genome or both. Thus, the bisexual reproduction can reappear more or less easily according to individuals, and an adaptative selection is possible.

* Intersexes or gynandromorphs: individuals showing female genitalia and 1 or 2 male antennae, appearing not only after heat or antibiotic treatments but also spontaneously in the thelytokous strains.

08-077

PARASITOID MEDIATED HORIZONTAL TRANSFER OF *WOLBACHIA* BETWEEN PARASITOID HOSTS, AND BETWEEN HOST AND PARASITOID.

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Wolbachia are a closely related group of intracellular α -proteobacteria which are widely distributed throughout the arthropod lineages and which exhibit vertical transmission (maternal) to successive host generations. the ability of these parasites to alter the host physiology (e.g. cytoplasmic incompatibility, parthenogenesis, feminisation and altered host immunity) has centred interest around the role of these symbionts in speciation or biological control. Molecular phylogenetic analysis of *Wolbachia* evolution and divergence relative to its arthropod hosts, along with recent observations in the spread of this parasite, strongly infers a role for horizontal transfer within and between arthropod species, at least in an evolutionary time scale.

Here we present experiments utilising both *Drosophila* and *Lepidoptera*-parasitoid systems, that strongly support a role for insect parasitoids as vectors for the horizontal transfer of *Wolbachia* between the parasitoid prey species, and also between the parasitoid and its prey, within an ecological time scale. However, parasitoid mediated horizontal transfer of *Wolbachia* displays a strong co-dependency upon the parasitoid's prey species and the *Wolbachia* isolate. The implications of this specificity of efficiency of horizontal transfer for host and parasite co-evolution and speciation will be discussed in the light of this data.

08-076

HOST AND PARASITE CO-SPECIFIC INTERACTIONS IN *WOLBACHIA*-MEDIATED INDUCTION OF CYTOPLASMIC INCOMPATIBILITY AND PARTHENOGENESIS IN *DROSOPHILA* HOSTS.

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Wolbachia are a closely related group of intracellular α -proteobacteria that induce altered host (arthropod) physiology. Following the isolation of *Wolbachia* free of host cell organelles and transfecting into different *Drosophila* hosts, the ability of *Wolbachia* to induce uni- or bidirectional cytoplasmic incompatibility (CI) or parthenogenesis was found to be dependent upon both the *Wolbachia* isolate and the *Drosophila* host utilised. Additionally, the same *Wolbachia* isolate give different effects in different geographical isolates of the same *Drosophila* species, as well as in different species. Moreover, the differences in degree and type of altered host physiology induced in the transfectants varied markedly both within and between the *Drosophila* groups studied (melanogaster, mercatorum / repleta and quinaria / testacea). These differences are not accounted for simply by the different parasite load between these transfecteds hosts (although this has a significant role) and thus, these data are consistent with both a host and parasite co-interaction. The implications for host and parasite co-evolution as well as horizontal transfer of the parasite between hosts will be discussed.

08-078

WOLBACHIA -MEDIATED CYTOPLASMIC INCOMPATIBILITY IN *DROSOPHILA*: COMPARISON OF PROTEIN PROFILES BETWEEN INFECTED AND UNINFECTED FLIES

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Wolbachia infections have been observed in various arthropod species from diverse orders, in most cases being associated with the phenomenon of cytoplasmic incompatibility. Cytoplasmic incompatibility is usually expressed when infected males mate with uninfected females. Eggs from such a cross do not successfully complete karyogamy. Since *Wolbachia* are shed from sperm during maturation, it is conceivable that *Wolbachia* condition the developing spermatid, and the conditioned sperm can be rescued exclusively in the eggs containing *Wolbachia*. In an attempt to better understand the molecular mechanism of cytoplasmic incompatibility, we studied the protein synthesis by *Wolbachia* in several *Drosophila* strains, and found a predominant *Wolbachia* protein whose molecular weight varied among strains. We also report the protein profiles of sperm from *Wolbachia* infected and uninfected male flies.

08-079

POSTMATING REPRODUCTIVE ISOLATION BETWEEN *CHRYSOPEA QUADRIPUNCTATA* BURMEISTER AND *C. SLOSSONAE* BANKS (NEUROPTERA: CHRYSOPIDAE)

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In laboratory tests, *C. quadripunctata* showed geographic variation in a postmating, prezygotic barrier to hybridization with its sister-species, *C. slossonae*. When paired with *C. slossonae* males, *C. quadripunctata* females from populations that are sympatric with *C. slossonae* had lower incidences of fertile oviposition than those from allopatric populations. Sperm was transferred to *C. quadripunctata* females in all the interspecific pairings, but lack of fertile oviposition was associated with sperm remaining in the bursa copulatrix, rather than reaching the spermatheca. The *C. quadripunctata* females that failed to lay fertile eggs when crossed with *C. slossonae* males invariably produced viable *C. quadripunctata* offspring (no hybrids) within one day after the heterospecific male was replaced with a conspecific one. Thus, we concluded that the barrier to hybridization may involve a species-specific interaction that influences movement of sperm to the spermatheca. When *C. slossonae* females were paired with *C. quadripunctata* males, the incidences of fertile oviposition were high and there was no apparent geographic variation in the degree of hybridization. As with *C. quadripunctata* females, unsuccessful hybridization of *C. slossonae* females was associated with retention of sperm in the bursa copulatrix. The patterns of inter- and intraspecific variation in hybridization indicate that *C. quadripunctata* harbors variation in the mechanism that controls sperm movement to the spermatheca and that the evolution of reproductive isolation between *C. quadripunctata* and *C. slossonae* may include natural selection for increased expression of this mechanism.

08-081

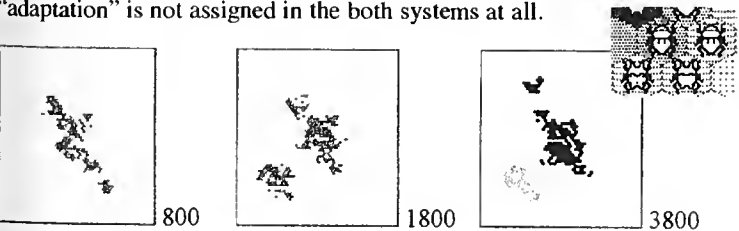
SPECIES PHENOMENA ON BIPARENTAL A-LIVES

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On the species phenomena, such as species entity, speciation event, diversity among local populations and intraspecific variety, biologists have proposed many models, concepts concerned and hypothetical visions. The validities of them, however, have been assessed simply by imagination subjectively. Exploiting the methods of simulation or A-Life, we are able to reconstruct the models, to observe the dynamics objectively and to evaluate or reject them fairly.

In our system (DEME, breeding A-Life species complex *Stigmaticon cornigera*; DEMEV, *S. mundana*; DEME(W), *S. fenestris*), the rules are derived from three fundamental dynamical principles, (1) individuals reproduce through bisexual interbreeding, (2) combinations of character states affect success or failure of the mating, and (3) the character states are inherited, but mutation may break out accidentally. The "biological concept of the species" is fully adoptable to these kinds of A-Lives. We can identify or discriminate individuals to the species observing the concept (definition) of species.

The system emerges well-nigh every species phenomena, the unity of species, the transition among generations, character differentiation in local populations of the species, formation of cline, allo- and parapatric speciation and hybrid zone. When the concept of "niche" is appended in the system (ECDM, *S. macacobia*), the sympatric speciation and the secondary sympatricity were observed. Note that the concept of "adaptation" is not assigned in the both systems at all.



08-080

EXTENSIVE MOLECULAR SIMILARITY IN SYMPATRIC FRUIT FLY SPECIES

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The morphologically similar species *Bactrocera tryoni* and *Bactrocera neohumeralis* occur in sympatry along the eastern seaboard of Australia. The distribution of *B. neohumeralis* is contained within the distribution of *B. tryoni*. The host range of the two species is very similar, and the two species have been known to emerge from the same piece of fruit. The basis for the maintenance of speciation appears to be mating time. Mating in *B. tryoni* occurs at dusk, whilst in *B. neohumeralis* mating occurs during the middle of the day.

Sequences from mitochondrial regions have been obtained for many individuals of each of the two species. These show a surprising level of homology, with shared polymorphic sites and similar average genetic distances within and between species. Following this finding, we searched for differences in non-coding nuclear DNA sequences and in the *period* gene which is involved in circadian rhythms. High levels of homology have been found for all regions studied.

Alternative hypotheses for the extensive homology are (1) very recent divergence of the two species, (2) high levels of introgression. Both explanations are difficult to reconcile with the maintenance of the mating dimorphism.

08-082

INVESTIGATION OF HYBRID DISGENESIS IN INDIVIDUALS FROM RECIPROCAL CROSSES BETWEEN POPULATIONS OF *Anastrepha fraterculus* (DIPTERA: TEPHRITIDAE) FROM SOUTHERN BRASIL.

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The fruit fly specie *Anastrepha fraterculus* is one of the pest in the fruit orchards in the southern Brasil. Several authors considering this species as a complex of cryptic species. This work analysed several aspects related with the specie's reproduction crossing two populations isolated. No occurrence of hybrid inferiority was found in egg-adult viability ($\chi^2=4.603$, DF=3) and hybrid disgenesis when the Kruskal-Wallis test was applied to detection of disgenic gonads considering the size and the number of ovocits per ovary [H=1.18; DF=3 (ovaries), H= 0.94, DG=3 (testicles) and H=3.38, DF=3 (ovocits). The results suggested that populations analysed are the same specie. This work starts the possibility of future investigations with crosses between populations located geographically more distant. Grants and fellowships: CNPq, ABPM.

08-083

REPRODUCTIVE ISOLATION OF PARAPATRIC *PONTIA DAPLIDICE* AND *P. EDUSA* (LEPIDOPTERA: PIERIDAE) IN A CONTACT ZONE

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The closely related parapatric butterfly species *P. daplidice* and *P. edusa* have a contact zone in Northern Italy, as we have shown previously. This contact zone is associated with a rather small hybrid zone, where both F₁- and backcross hybrids are observed at low frequencies with strong deviation from Hardy-Weinberg equilibria, as revealed by enzyme electrophoresis using four diagnostic loci. These butterflies are considered medium distant migrants. However, there is no evidence of gene flow beyond the hybrid zone as shown by the allozyme data.

We analyse parameters that potentially account for the reproductive isolation of the butterfly species concerned. These include estimates of dispersal with capture-mark-release techniques, spermatophore counts, analysis of selective mating with allozyme techniques, and comparison of mortality and rates of development under laboratory conditions (30°, 17L:7D, using *Reseda lutea* as food plant) of larval instars and pupae among F₁- and backcross hybrids as compared to the pure forms.

08-085

GENOTYPIC STRUCTURE AND REPRODUCTIVE ISOLATION BETWEEN SYNTOPIC POPULATION OF *CHALCOLESTES VIRIDIS* AND *C. PARVIDENS* IN CENTRAL ITALY (ODONATA: LESTIDAE)L. Dell'Anna¹, E. De Matthaeis¹, M. Cobolli² & C. Utzeri¹¹ Dip.to Biologia Animale e dell'Uomo, Università "La Sapienza", Roma, Italy² Dip.to Scienze Ambientali, Università di L'Aquila, Italy

Chalcolestes viridis and *C. parvidens* have been recognised as separate species quite recently (Cobolli et al., 1994. Atti 17° Congr. naz. ital. Ent., pp. 77-82). The two taxa were distinguished on the basis of the male's superior abdominal appendages (the cerci), while it was not possible to separate either females and the preimaginal stages. In a pond in Castel Porziano, Roma, Italy, both species coexist, generating pure and hybrid offspring. An investigation on genetic structure, morphology and ecology of the two species was carried out to evaluate the amount of hybrid specimens in the syntopic area and to identify the pre-mating isolating barriers between the two species. A morphometric analysis (total body length) of samples from the syntopic and allotopic areas (Greece, Spain) has shown that in syntopy, the males of *C. parvidens* are significantly shorter than those of *C. viridis*, and the same is valid for the females which were pairing with the males of the two species. Also in syntopy, the two species show peaks of activity at different times in the day. As to allotopic populations, neither body length nor activity times differ. In hybrid specimens, either the shape of anal appendages can be intermediate between those of genetically pure specimens, or morphological characters of the two species can coexist. The genotypic structure was investigated by means of electrophoresis of multilocus enzymes. The average genetic distance between the two species is $D_{NEI} = 0.586$. The two species differ at 7 diagnostic loci out of 16 checked. Hybrid specimens were heterozygous at either all loci (F₁-hybrids) or some of the diagnostic loci. The former showed a mixed morphology, while the latter could show parental morphology (particularly so those which were heterozygous at a single locus). The overall percent of F₁-hybrids in the overall sample (N = 131) was 4.6 %, which matches the percent of interspecific pairs (4.7 %) in the same area (females belonging to each of the two species were identified by electrophoresis). Despite the occurrence of some hybridisation, which witnesses some genetic and morphological compatibility, the two species maintain isolated by such barriers as different activity times and different shape of anal appendages.

08-084

SPECIATION IN GREEK BUSHCRICKETS OF THE *POECILIMON PROPINQUUS*-GROUP (ORTHOPTERA, ENSIFERA, PHANEROPTERIDAE)

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The mechanisms which lead to the evolution of new species are not well understood. The most powerful explanation originate from Mayr and is known as the "Biological Species Concept" (BSC). According to this concept most species evolved in allopatry. For secondary contact the "BSC" predicts that character displacement should be a common event, but very few cases of character displacement are known. This problem lead to the formulation of alternative concepts. In this contest I have tested the prezygotic isolation in six species of the *Poecilimon propinquus*-group. These species are parapatrically distributed in Greece. Barriers against hybridisation may consist of song or structure of male cerci. The mating behaviour between different species were observed in 27 interspecific pairing and crossing experiments using 600 males and females. Neither the song discrimination by females nor the fit of male cerci to female basal fold show evidence for reinforcement. I present an alternative explanation for song discrimination by females.

08-086

ANALYSIS OF THE MITOCHONDRIAL GENOME OF FLIES THAT CAUSE MYIASIS BY POLYMERASE CHAIN REACTION AND RFLP.

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Animal mitochondrial DNA (mtDNA) has been used extensively in studies of evolutionary and population genetics as well as in systematics of closely related species. More recently, an emphasis has been given on overall mitochondrial genomic structure. The advent of the polymerase chain reaction (PCR) with the use of universal mtDNA primers permits the sequencing of the DNA of species for which no previous sequence information exists.

Flies that cause myiasis from Calliphoridae and Cuterebridae families are all obligate or facultative parasites in the Neotropical region. Blowflies are typically necrophagous and saprophagous, and are the major initial decomposers of animal carcasses. The New World screwworm fly, *Cochliomyia hominivorax* and the bot fly *Dermatobia hominis* are the obligate ectoparasites considered the most important pests of warm-blooded livestock in Brazil. However, it also parasitizes wild animals and humans.

At the genetic level, relatively little is known of the molecular markers to estimate intra and interspecific variability of Brazilian blow flies and bot fly.

This study reports the characterization of mtDNA regions of six dipteran species causing myiasis: *Cochliomyia hominivorax*, *Cochliomyia macellaria*, *Chrysomya megacephala*, *Chrysomya albiceps*, *Phaenicia eximia* and *Dermatobia hominis* using the PCR of the control region (called the A+T rich region in insects), the Cytochrome Oxidase genes subunit I and II (COI/II), the rRNA 12S and 16S and the Cytochrome b region. The A+T rich region varies in length from 1200 to 1800 nucleotides for these species. The differences in the mtDNA control region observed by PCR were useful to identify species-specific markers in the amplified DNA. Diagnostic restriction sites with *TaqI* in COI/II recognized three genera, *Cochliomyia*, *Phaenicia* and *Dermatobia*. Both regions are particularly well suited as molecular markers for species identifications and are indicative of variable and conservative sites in the mtDNA molecule.

These results suggest a size variability for A+T rich region among the six studied species and were also useful to predict diagnostic restriction sites for COI and COII regions. The PCR-RFLP technique applied to characterize these dipteran species should provide material for their further analysis both structurally and functionally and their comparisons with other insect mtDNAs.

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08-087

MITOCHONDRIAL DNA LENGTH VARIATION IN THE PEA APHID
ACYRTHOSIPHON PISUM.

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The genetic relationships among 29 European clones of the pea aphid were investigated by mitochondrial DNA RFLP, using a modified alkaline lysis technique. Genetic variation at restriction sites was not found, reflecting the generally low variation found in aphids. However, one restriction site was able to distinguish all these European clones from pea aphids studied in N. America.

Length variation (which may correspond to variable tandem repeats) was found at 2 regions of the mitochondrial DNA molecule. Three size classes were obtained at region 1, which contained the A+T-rich region; and four size classes at region 2 which is located at the ND3 to ND5 region of the molecule. There was no association between variation at the two regions of the molecule. The different length variants were not correlated with host-plant affiliations of the aphids.

08-089

RANGEWIDE VARIATION OF MITOCHONDRIAL DNA AMONG
POPULATIONS OF THE MARITIME PINE SCALE
MATSUCOCCUS FEYTAUDI DUC. (HOMOPTERA:
MARGARODIDAE)

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Matsucoccus feytaudi is a specific pest of maritime pine (*Pinus pinaster*). Western populations of the insect are endemic whereas eastern populations are thought to be recently introduced. They developed severe outbreaks in Southeast France and are presently spreading in the north of Italy. A future epidemic evolution is also feared in Corsica where a scale population has just been discovered by pheromone trapping. The relationship between potential outbreaks and the origin of *M. feytaudi* populations was thus investigated by studying the phylogeography of the pest. Scales were sampled over the maritime pine natural range. Mitochondrial DNA of single minute males was amplified using PCR, and analysed with the restriction fragment length polymorphism (RFLP) and single strand conformation polymorphism (SSCP) techniques. Preliminary results are presented, which precise the origin of the newly discovered populations and outline the evolutionary history of this pest.

08-088

MITOCHONDRIAL DNA HAPLOTYPES AND QUATERNARY RANGE
CHANGES OF *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE)

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The phlebotomine sandfly *Phlebotomus* (*Phlebotomus*) *papatasi* has an extensive geographical range in the subtropics of the Old World, extending from Portugal and Morocco to the Indian subcontinent, and is found on many of the larger Mediterranean islands. It occurs at altitudes from sea-level up to 2100m, and geographical populations display varying degrees of diapause (overwintering as fourth instar larvae). However, it is only abundant in semi-arid and arid bioclimatic zones and is rarely collected in northern Spain, southern France and northern Italy. As such, it is believed that its range contracted considerably during Quaternary glacial periods. Southern hybridization analysis with homologous probes, the (direct) sequencing of PCR products and PCR-RFLP analysis have all been used to characterize mitochondrial haplotypes found in 15 populations of *P. papatasi* from 10 countries, from Spain and Tunisia in the West to Afghanistan and India in the East. Comparative (phylogenetic) sequence analyses have been used to relate these populations and those of other *Phlebotomus* species and to postulate the geographical range of *P. papatasi* from the late Quaternary back to the Miocene-Pliocene, when we conclude it diverged from the morphologically and ecologically similar *Phlebotomus* (*Phlebotomus*) *duboscqi* which is now restricted to the sub-Saharan Sahel. This project is supported by grants from the British Council (Tunis Office) and the CEC/EU (Brussels).

08-090

GENETIC VARIATION IN GEOGRAPHIC POPULATIONS OF THE SCREWORM
FLY (Diptera: Calliphoridae), FROM BRAZIL.

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Screwworm, *Cochliomyia hominivorax* is an obligate ectoparasite causing severe myiasis in warm-blooded livestock, wildlife and humans throughout the Neotropical Region. The genetic analysis based on cytogenetics and allozyme loci studies have been conducted previously in several geographic populations of screwworm flies from North and Central America. The results obtained show no evidence of reproductively isolated populations, suggesting that *C. hominivorax* is a single, highly polymorphic species. In this work, cytogenetic and mtDNA analysis by RFLP were used to examine genetic variation and population structure of screwworm flies in seven populations from five Brazilian States: Amazonas, Bahia, Minas Gerais, São Paulo and Rio Grande do Sul. Six different karyotypes (2n=12) were identified through morphologic analysis of metaphase chromosomes with Giemsa and Quinacrine staining. The main karyotypic interpopulational variation refers to the length and morphology of the chromosomes, the location and numbers of secondary constrictions of autossomes II, III and VI and the sex chromosomes. The Q-band showed differences in the intensity and location of the fluorescent regions in pairs II, III and sex chromosomes. Cytogenetic studies have demonstrated the existence of interpopulational variation but are unable to unambiguously resolve screwworm fly population structure. Restriction enzyme analysis of mtDNA variation is a likely tool to study intraspecific population structure in screwworm fly from Brazil because of considerable polymorphism as was demonstrated in previous work conducted in five populations in São Paulo State. Here, we extend that analysis by means of an increased number of geographic screwworm flies populations. The total DNA of 520 individuals sampled was digested with 15 restriction endonucleases and probed with five cloned *Hind* III fragments of *C. hominivorax* mtDNA. The survey revealed that five enzymes *Clal*, *Hae*III, *Hind*III, *Msp*I and *Pvu*II were suitable to detect mtDNA variation among the populations. Based on the fragment patterns obtained for these five enzymes, a total of 14 haplotypes in combination was detected. Estimates of mtDNA sequence divergence (d) between haplotypes ranged from 0.015 to 0.040. The cladogram of the geographical distribution among the observed haplotypes suggests that the sampled screwworms probably belong to a single evolutionary lineage with populations interconnected by reduced gene flow.

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08-091

A PHYLOGENY OF APHID PARASITIDS (HYMENOPTERA:APHIDIIDAE) BASED ON DNA SEQUENCE OF MITOCHONDRIAL 16S rRNA AND NADH1 DEHYDROGENASE GENES.

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The hymenopteran family Aphidiidae consists of about 50 genera and more than 400 described species, which parasitize exclusively aphids. The importance of aphidiids stems from the fact that they are important biological control agents of aphid pests throughout the world. Various hypothesis concerning the generic- and species-level relationships, based on morphological characters exist. In this poster, a phylogeny for 15 genera and 45 species of aphidiids based on DNA sequence analysis of portions of the mitochondrial 16S rRNA and NADH1 dehydrogenase genes will be presented. The results of the analysis generally confirmed the existing hypotheses concerning generic-level relationships. The implications of the molecular phylogeny to the evolution of pupation habit (inside vs. outside the host) and host range (monophagy vs. oligophagy) will be addressed. Supported by USDA-NRI grant 9401865 to SK and MM.

08-093

HOST-PLANT RELATIONS OF *OSTRINIA NUBILALIS* AND FACTORS OF EVOLUTION IN THE GENUS *OSTRINIA* (LEPIDOPTERA: PYRALIDAE)

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The European corn borer (ECB), *Ostrinia nubilalis*, and allied forms are shown to be characterized by complex population structure adaptively connected with the general ecological features: humidity conditions, thermic regimes, and host plants. Depending on the action of the factors mentioned, borers had formed genetic adaptations which allowed them to exist in wide and differentiated life space (Frolov, 1994 a,b).

Occupation of host plants by ECB seems to be performed in two different strategies of host exploitation: i) species-generalized but stages-specialized (feeding occurs on mono- and dicotyledonous hosts at stages near flowering), and ii) species-specialized but stage-generalized (few monocotyledonous species infested at wide range of their stages). The existence of strategies is supported by ethological isolation (Frolov, 1994 b,c). Certain level of specificity in host-plant relations with maize, sorghum and millet was observed among populations following the second strategy of host exploitation.

The study was in part supported by grants from the ISF (NTH000 and NTH300).

08-092

GENETIC VARIATION IN MITOCHONDRIAL DNA OF *COCHLIOMYIA MACELLARIA* (DIPTERA:CALLIPHORIDAE) FROM BRAZIL

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Cochliomyia macellaria belongs to an endemic New World genus and ranges from southern Canada to Argentina, but it is especially abundant in the tropical regions. It is a carrion breeder which is a secondary agent of myiasis and a purely saprophagous species, which is perhaps the most widely distributed calliphorid in the Americas. This species has been implicated as a mechanical vector of human and animal diseases. Recently, the Western Hemisphere has been invaded by four Old World blow flies of the genus *Chrysomya* (Robineau-Desvoid). These species, particularly *Chrysomya albiceps* and *C. putoria*, occupy the habitats of the native species, including *C. macellaria*, providing interspecific competition, and have caused a population reduction and movement of *C. macellaria* in several regions of Brazil and Peru.

At the genetic level, investigations of natural populations of *C. macellaria* have been limited to karyotypic characterization. Investigation of genetic variability among populations of *C. macellaria* would help understand historical patterns of dispersal and current levels of gene flow.

In this report restriction fragment analysis (RFLP) of mitochondrial DNA (mtDNA) was used to examine genetic variation of six populations of *C. macellaria* from Brazil. The total DNA of 371 individuals were digested with 13 restriction endonucleases and probed with the entire mitochondrial genome of *Cochliomyia hominivorax*. Five endonucleases, *EcoRV*, *HindIII*, *MspI*, *PvuII* and *XhoI*, detected polymorphism, yielding thirteen haplotypes in combination. Nucleotide divergence estimates (d) between pairs of haplotypes ranged from 0.2% to 10%. Restriction site heteroplasmic individuals were found in two analysed populations. These results have demonstrated variability in mtDNA of this species in Brazil and revealed the existence of a probable "refuge" of this blow fly in the Amazon region.

Financial support: CAPES, FAPESP and CNPq.

08-094

SEQUESTRATION OF A LARGE VARIETY OF TOXIC SECONDARY PLANT METABOLITES BY CLOSELY RELATED SAWFLY SPECIES

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Sawfly larvae of the tribe Phymatocerini are specialists on one plant taxa such as *Veratrum*, *Polygonatum*, *Iris*, *Helleborus*, *Ranunculus* or Poaceae. A sequestration of alkaloids from *Veratrum* is known to occur in one sawfly species. The question is whether sequestration is a general phenomenon for the Phymatocerini. It would bring us to a rare situation where closely related insect species have to deal chemically with a large variety of secondary plant metabolites which possess toxic properties. In the plant genera mentioned above, steroidal alkaloids, quaternary alkaloids, steroid saponins, spiro-bicyclic triterpenoids, and lactones are major chemical types. Most of these chemicals are known to have a toxic effect on vertebrates and/or invertebrates. During a field season in Switzerland, eight Phymatocerini species were collected. The integument of the larvae was wounded easily, and disturbed larvae remained immobile. Moreover, the hemolymph of the larvae, tested on ants, proved to act as a feeding deterrent. By chemical analyses, the hypothesis that sequestration could occur recurrently in several species was investigated. I discuss from an evolutionary point of view the question whether sequestration could have evolved as a trait related to the high toxicity of secondary plant metabolites.

08-095

WAS THERE A COEVOLUTIONARY RACE BETWEEN THE STAR ORCHID *ANGRAECUM SESQUIPEDALE* AND THE HAWKMOTH *XANTHOPAN MORGANI PRAEDICTA* ?

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Sphingophilous flowers are adapted to engage hawkmoths for their pollination needs by evolving long tubes or spurs with deeply situated nectaries exclusively accessible for hawkmoths. A famous and extreme example of such a flower is *Angraecum sesquipedale*. Darwin (1862) hypothesized, that this flower must have evolved the long spur as an adaptation to the long tongue of a hawkmoth and that the tongue on the other side must have increased its length in the course of an evolutionary race. This idea of coevolution has recently been emphasized (Nilsson 1988). There is however, evidence only for the advantage of spur elongation for increasing the orchid's fruiting success (Nilsson 1988) - not for the reciprocal increase in tongue length.

Investigations in the field and flight cage support the hypothesis, that the typical specializations of the hawkmoths are adaptations against predation: As generalist feeders most hawkmoths visit a variety of less specialized flowers. By their long tongue combined with hovering flight, hawkmoths keep distance to the flower during nectar uptake and diminish the risk of being captured especially by ambushing predators e. g. spiders. By shortening the stay time ("flower jumping"), often combined with incomplete flower exploitation, the moth can further reduce the danger of being captured. The Malagasy hawkmoths with the longest tongues of the genera *Xanthopan*, *Coelonia* and *Agrilus* exhibit a "swing-hovering" flight which enables them to fully deplete "normal" flowers without the necessity to stay on the spot and thus renders a predator's attack almost impossible.

The pollination of *Angraecum* by *Xanthopan* is described for the first time and the mechanism by which the flower prevents the hawkmoth from "swing-hovering". A model is presented which explains the evolution of extremely long spur of this angraecoid orchid on the basis of (anti-predator-) preadapted hawkmoths instead of Darwins model of a coevolutionary race.

08-097

ENVIRONMENTAL FACTORS INFLUENCING THE VISITS OF *TETRAGLOSSULA* SPP (HYMENOPTERA: COLLETIDAE) TO THE FLOWERS OF *LUDWIGIA ELEGANS* (ONAGRACEAE)

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The interaction between the flowers of *Ludwigia elegans* and *Tetraglossula* spp was studied in three areas in São Paulo, Brazil (Mairinque, MA, Campos do Jordão, CJ, and Ribeirão Preto, RP), in relation the morphological, behavioural, phenological and temporal aspects.

Tetraglossula bigamica (RP) and *Tetraglossula ventralis* (MA and CJ) were the most frequent species on the flowers. They were seen from December to May, when *L. elegans* shows its blossoming peak.

During its months of activity, *Tetraglossula* spp showed a circadian rhythm of pollen collection synchronized with the opening of the anthers of *L. elegans*. These rhythms were probably influenced by the light/dark cycle, as they did not occur in the night and by the environmental temperature, because interaction was not observed below 18 °C at the three areas.

The females of *Tetraglossula* spp collected pollen frantically rubbing the abdomen against the anther. Pollen was kept in the rigid, long, and simple hairs of the abdominal scopa.

From the results it may be concluded that *Tetraglossula* spp were the most efficient pollinators of *L. elegans* due to their frequency of occurrence and morphological, behavioural, and temporal adaptations. The light/dark cycle and environmental temperature seem to play important roles in the interaction, providing daily synchronization and annual adjustments between bees and flowers.

Support: CAPES, CNPq and FAPESP.

08-096

MEXICAN DIABROTICITES (COLEOPTERA: CHRYSOMELIDAE) AND CUCURBITACEAE: AN EXAMPLE FOR CHEMICALLY MEDIATED INSECT-PLANT COEVOLUTION?

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Choice tests were conducted to determine relative degree of specialization of feeding behavior of 8 Mexican diabroticite species in the genera *Diabrotica* and *Acalymma* (Chrysomelidae: Luperini). Adult beetles were offered a choice between cotyledons of a non cucurbitacin-containing cucurbit, corn and beans. In a second assay a cucurbitacin-containing (bitter) cucurbit was added to the array of plants offered. All species showed distinct preferences in the first assay. When the bitter cucurbit was included, all species tested changed their host choice and significantly preferred the bitter cucurbit. This observation led to another series of choice tests, offering a non cucurbitacin-containing cucurbit and 2 cucurbits which contained either one of the 2 primary types of cucurbitacin. All species significantly preferred the bitter over the non bitter cucurbits and cucurbitacin B over cucurbitacin E-containing cotyledons.

These results indicate that the association of diabroticites with Cucurbitaceae is partially mediated by plant secondary compounds.

The role of cucurbitacins for the hypothesized coevolutionary association of diabroticites with cucurbits and for the evolution of oligo- and polyphagy in diabroticites is discussed.

08-098

SYNCHRONISATION BETWEEN HESPERIID BUTTERFLIES (INSECTA: LEPIDOPTERA) AND THEIR NECTAR PLANTS

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Five skipper species (Lepidoptera: Hesperidae), *Thymelicus sylvestris*, *T. lineola*, *T. acteon*, *Ochlodes venatus*, and *Hesperia comma*, their nectar plants and preferences were investigated in summer 1994. The study areas were dry and wet meadows and a forest in Middle Hesse at about 200 to 350 m above sea level. The periods of blooming time and the abundance patterns of the nectar plants were determined.

The abundance patterns of the skipper species were recorded on transects. Evidence was found for a synchronous phenology between the skipper species and some of their frequently visited nectar plants. Peaks in the flight period of the skippers correlate with peaks of the blooming time of the special nectar plants. At some sites syntopically flying *Thymelicus*-species show differences in the choice of their most frequented nectar plants that correlate with differences in their phenology. Possible causes of the synchronisation are discussed with special regard to a co-evolutionary relationship, niche-choice and energy balance. Potential implications for management practices (e.g. unfavourable grazing or mowing times) are discussed.

08-099

POLLINATORS AND VISITING INSECTS OF INFLORESCENCES OF THE *EUTERPE OLERACEAE* MART. (ARECACEAE) IN EASTERN AMAZONIA.

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This study evaluates the floral biology of "açai" palm tree (*Euterpe oleraceae* Mart.) in a natural population located on Combu Island (25° 48' W, 1° 25' S), Pará, Brazil. The observations were carried on from January to December, 1991. The results showed that *E. oleraceae* is a monoic, dicogamous and protandric species. The bracts open five days after maturation. The male flowers remain opened between 10 and 12 days in the inflorescences. The female flowers open after the male flowers have fallen completely and remain receptive during five days. Both male and female present diurnal anthesis. The insects observed visiting the inflorescences belong to the following orders: Coleoptera (11 species), Hymenoptera (4 species), Diptera (3 species) and Homoptera (1 specie). The possible pollinators were 4 species of beetles.

08-101

THE IMPACT OF REED BED MOWING ON THE GENETIC STRUCTURE OF *Lipara lucens* POPULATIONS.

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Reed beds are detritus-based ecosystems. Every year large amounts of litter accumulate which results in the drying out of the ground surface. This can cause major conservation problems for the reed beds and its inhabitants. Mowing is one of the main management methods used to reduce litter input. Although this practice conserves the reed bed for drying out, it can strongly influence the population structure of invertebrate animals by causing a drastic reduction of population numbers. Especially endophagous herbivores such as gall-forming species, whose larvae develop in the above ground situated plant structures, are assumed to be highly vulnerable.

Here we study the genetic consequences of mowing on *L. lucens*, a strict monophagous gall-forming parasite of the common reed *Phragmites australis*. We compared the genetic structure of six populations of mown and unmown parts of two large reed beds near Antwerp, Belgium. Mowing during late winter or early spring, with removal of the litter, causes almost complete extinction of *L. lucens* populations. Although recolonization occurred fast during the present study (the following year), genetic population structure is clearly affected. We found a consistent departure from Hardy-Weinberg equilibrium in populations from mown reed beds. Furthermore, mowing leads to a drastic decline in genetic variability.

08-100

THE ROLE OF DISTANCE AND HOST-PLANT ASSOCIATIONS IN THE DIFFERENTIATION OF *TETRANYCHUS URTICAE* POPULATIONS (ACARI: TETRANYCHIDAE). A MULTILOCUS ALLOZYME ANALYSIS.

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Tetranychus urticae Koch is a phytophagous mite with a world-wide distribution. It can reach high densities causing important damages on open field and greenhouse cultures. Few studies on the genetic variability of this species have been undertaken mainly due to their minute size. We have used an isoelectrofocusing electrophoresis method on several stacked cellulose acetate membranes as support, which allowed to reveal 4 enzyme systems on single females.

Based on the combined information of four loci, we showed that in Crete (Greece), there was no differentiation between populations of *T. urticae* colonizing different host-plant species (citrus, weeds, tomato, pumpkin and okra) within an area of 50 m². In contrast, populations separated by 150 m or more were significantly different. These results are discussed in relation to the known patterns of migration in *T. urticae*.

08-102

REPELLENT EFFECTS OF HERBIVORE-INDUCED MAIZE VOLATILES ON THE CORN-LEAF APHID, *RHOPALOSIPHUM MAIDIS* (FITCH).

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Herbivory by caterpillars is known to induce a reaction in maize plants that results in the emission of a specific blend of volatiles by these plants. The volatiles are highly attractive to natural enemies of the herbivores, but little is known about the effects that the volatiles may have on herbivores themselves.

In laboratory and field studies we investigated the effects of induced maize volatiles on the behavior of winged and wingless morphs of the corn-leaf aphid, *Rhopalosiphum maidis*. For these experiments, maize plants were induced to emit volatiles by mechanically damaging certain leaves and by treating the damaged sites with regurgitate of the Egyptian leafworm *Spodoptera littoralis*. Subsequently, aphids were offered a choice between such treated plants and plants that were left unharmed. In a Y-tube olfactometer as well as in a field experiment, it was shown that, especially the winged aphids, had a preference for healthy, untreated plants.

We propose that odors that maize plants emit after an attack by herbivores may repel aphids because: 1) the odors may indicate the presence of competitors, 2) the odors may indicate the production of defense compounds by the plant, 3) the odors may make the plant more attractive to natural enemies of the aphid, and 4) one of the main compounds in the odor blend is the known aphid alarm pheromone, E-(β)-farnesene.

08-103

CONGRUENCES IN PILOSITY BETWEEN SPECIES OF *OPHRYS* (ORCHIDACEAE) AND THE FEMALES OF THEIR HYMENOPTERAN POLLINATORS (APOIDEA)

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Flowers of the mediterranean genus *Ophrys* offer hymenopteran males a set of sensations by imitating stimuli produced by their females. Attracted olfactorily and visually, the male alights on the orchid labellum and attempts copulation. Tactile cues such as the pilosity pattern and shape of the labellum guide the male to the correct position for pollen transfer. Hair structures, their orientation, density, length and arrangement in specific patterns of 27 *Ophrys* species and the females of their pollinators were investigated by using light and scanning electron microscopy. The function of specific hair patterns in positioning the males correctly on the labellum were tested in field experiments. Although hair structures of orchids are not in detail similar to those found on hymenopteran females, they are arranged and orientated alike on corresponding patches. Ethological studies showed that hair structures and distributions as those of the centre part of the labellum mimic the visual appearance of the female whereas the pilosity of the edge and the tip of the labellum lead to right orientation within a fraction of a second.

08-105

DOES AN *OPHRYS* PLANT CHEAT A POLLINATING MALE BEE MORE THAN ONCE?

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Ophrys spp. orchids are mostly pollinated by male bees, which are lured to the orchid by visual cues and volatile semiochemicals. At close range, chemical signals from the flowers release sexual behaviour in the males, acting in a manner similar to the sex pheromones from females. The male bees try to copulate with the flower labellum and thereby transfer pollen from one flower to another. Our field observations show that male pollinators are very rare on *Ophrys* plants. Therefore, strategies that results in males visiting more than one flower in the same inflorescence would be expected to evolve in plants in order to optimise pollination events. Since male bees are able to learn the distinctive odour bouquets of individual females during mating attempts, and use this information to avoid females they have previously mated, one would expect the odour bouquets of *Ophrys* flowers to vary within populations. The aim of our work was to determine whether flower-specific olfactory recognition signals occur in the orchid *Ophrys sphegodes* by conducting 1) behavioural learning experiments with the pollinating male bees of *Andrena nigroaenea* and 2) quantitative chemical analyses of the headspace odours from individual flowers.

Our data from the behavioural tests indicate that male bees learn the odour bouquets of individual flowers during mating attempts and recognise them in further encounters, since they avoid trying to mate with flowers they have visited previously, but not with other flowers, either on the same or on a different plant. Gas chromatographic analyses of individual flower odours showed that the floral volatile bouquets differed between plants as well as between flowers within an inflorescence. We assume that variation in the odour bouquets of individual flowers within an inflorescence raises the chance of more than one flower being visited by the same male and thereby influences the plants pollination success and individual fitness.

08-104

VARIATION OF THE POLLINATOR ATTRACTING ODOR SIGNAL OF *OPHRYS SPHEGODES* SUBSP. *SPHEGODES* MILLER.

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The Mediterranean genus *Ophrys* is pollinated by sexually excited male Hymenopterans. Its floral scent is the most important cue for eliciting male mating behavior. Although behavioral experiments showed that male bees are able to learn individual odor bouquets of females as well as of *Ophrys* flowers, none of the recent investigations on *Ophrys* scent is concerned with variation of scent emission of single flowers. When single flowers were offered to male bees, a variation of the attractiveness was observed which was probably due to variation of the odor bouquets. We investigated the composition of scent of single *O. sphegodes* flowers, its alteration after pollination, and nocturnal floral scent emission by means of headspace sorption. Odor samples were analyzed by mass spectrometry and gas chromatography.

32 compounds were identified within the volatile bouquets. Aldehydes were the most abundant class of compounds (40-50 %). There was no decrease in nocturnal scent emission, but bouquets collected during day and night were significantly different. Two to four days after pollination, flowers emitted significantly different odor bouquets, and the total amount of scent was significantly lower compared with unpollinated flowers. Preliminary behavioral tests where synthetic compounds were offered to the pollinator bees indicated a certain attractiveness of 2-nonanol, which was emitted in lower amounts and proportion after pollination. Changes within odor bouquets after pollination may lead to lower attractiveness of pollinated flowers, thus guiding subsequent pollinators to unpollinated flowers of an inflorescence.

08-106

MICROHABITAT SEGREGATION OF GUILDS OF NEOTROPICAL ITHOMIINE BUTTERFLIES (NYMPHALIDAE: ITHOMIINAE) AND THE EVOLUTION OF MIMICRY

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Müllerian mimicry theory predicts that there should be convergence among the unpalatable species in an area to give a single aposematic colour pattern. Thus, the observed sympatry of many different butterfly mimicry complexes throughout much of the Neotropical forests requires explanation.

Evidence is mounting that sympatric mimicry complexes dominated by unpalatable Ithomiinae (ithomiines) and also Heliconiinae (heliconiines), are spatially segregated by microhabitat. In this paper data are presented which confirm that sympatric ithomiine complexes are segregated vertically by flight height. Flight height of ithomiines was found to be positively correlated with the height of their larval host-plants. Thus members of a mimicry complex utilise host-plants of similar heights. Flight height and host-plant height were also found to be positively correlated for non-mimetic British woodland butterflies, which suggests that this relationship is independent of mimicry. These and other data indicate that host-plant partitioning between butterfly species in a community results in the formation of microhabitat guilds of species. Mimicry complexes are microhabitat guilds, which suggests that mimicry has evolved between species which share a guild and not between guilds, perhaps through the action of microhabitat-dependent selection.

08-107

HEMOCYTES DNA AMPLIFICATION: A SUITABLE TOOL TO INVESTIGATE NUCLEIC ACIDS ON LIVING INSECTS

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Interestingly enough, purifying DNA from insects hemolymph seems not at all popular to date although a fast and reliable method of sampling DNA without sacrificing the insects could find a broad application in basic and applied research, i.e. population genetics, molecular genetics, parasitology, agricultural entomology, environmental mutagenesis only to mention a few. The hemolymph or blood of insects is a fluid plasma characterized by different types of nucleated cells in suspension. These cells are involved in different processes as tissue formation, storage, phagocytosis and metabolism. Hemolymph contains various inorganic ions determining pH values usually ranging from 6.0 to 7.7 with cell concentrations variable in different species. We pointed out a friendly and very fast procedure for the isolation from hemocytes of genomic DNA suitable for PCR reactions and we assayed this new procedure on insects belonging to different species as the cave cricket *Dolichopoda laetitiae*, the grasshopper *Anacridium aegyptium*, the beetle *Tenebrio molitor* and the leaf insect *Exstatosoma tiaratum*. The DNA obtained from hemolymph samples as small as 5 µl has been employed directly in PCR assays on both nuclear and mitochondrial sequences. This technique is particularly suitable when it is important to preserve viability of individuals; in our laboratory we are applying this methodology to studies concerning evolutionary relationships of cave cricket populations belonging to the genus *Dolichopoda*.

08-109

EVOLUTION-GENETIC ANALYSIS OF THE PALEARCTIC ANOPHELES MACULIPENNIS (DIPTERA: CULICIDAE) COMPLEX

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The phylogenetic relations and trend of species formation at 8 species of holarctic fauna of *Anopheles maculipennis* Complex were determined on the basis of study of linear and spatial (three-dimensional) organization of polytene chromosomes. The fundamentally new taxonomic index - architecture of polytene chromosomes of generative tissue (ovarian trophocytes) - was revealed. One allows to conduct diagnostics of any species including homosequential groups of species: *An. labranchiae* Fall. - *An. atroparvus* Van Thiel and *An. maculipennis* Mg. - *An. subalpinus* Hack. et Lewis. The peculiarities of molecular structure and polymorphism of heterochromatin at species of *An. maculipennis* Complex were studied. The adaptive features of chromosomal inversion polymorphism in *An. messeae* Fall. were cleared up. The morphological peculiarities of new species - *An. beklemishevi* Stegn. c. Kab. - were revealed.

08-108

SATELLITE DNA SEQUENCE VARIATION IN THE GENUS PIMELIA (COLEOPTERA, TENEBRIONIDAE).

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Several species of *Pimelia* from the Canary and Balearic Islands, and from mainland Spain show a highly conserved satellite DNA. This satellite DNA consists of a monomeric unit of 356 bp which is roughly 65% A-T rich. The sequence displays many clusters of three or more A-T residues, which can be related with DNA left superhelix curvature, as demonstrated by their retarded mobility in non-denaturing polyacrylamide gels.

These satellite DNAs have been taken as probes for fluorescent *in situ* hybridization (FISH) on meiotic and mitotic chromosomes where they show binding signals at the pericentromeric regions of all chromosomes, in agreement with the location of C-bands.

The species-specific satellite DNA sequences of *Pimelia* allow to study the phylogeny within the genus and make possible to compare this phylogeny with that based on the mitochondrial DNA.

08-110

HEMOCYTES: A GOOD SOURCE OF MITOTIC CHROMOSOMES FROM CIRCULATING CELLS OF INSECTS.

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Hemolymph of insects is a fluid plasma characterized by different types of nucleated cells in suspension. These cells are involved in different processes as tissue formation, storage and phagocytosis. In this study we investigate the mitotic property of hemocytes and report an easy and quick method to obtain mitotic chromosomes from hemocytes of insects. We have experimented this method on two different genera: *Dolichopoda* cave crickets and *Exstatosoma* leaf insects. This method is based on the mitotic property of circulating hemocytes so that it is sufficient to pick up few microliters of hemolymph to obtain mitotic chromosomes. After staining with Giemsa solution and with etidium bromure chromosomes were observed by means of optical microscope and by means of a confocal microscope. This method could be particularly usefull in studies of environmental mutagenesis, agricultural entomology conservation biology and evolutionary biology being possible to analyze the mitotic chromosomes of any individual keeping it alive.

08-111

CHROMOSOME SPECIATION IN PODISMINI GRASSHOPPERS (ORTHOPTERA, ACRIDIDAE) ON SAKHALIN AND THE KURIL ISLANDS

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The inversion in the X-chromosome of *Podisma sapporensis* from Sakhalin (Krylion population) ($2n\sigma=23$, NF=24, sex determination is $XO\sigma/XX\sigma$) and that of the translocation of autosome-X-chromosome in the population of this species ($2n\sigma=22$, NF=23, sex determination is neo- $XY\sigma$ /neo- $XX\sigma$) from Golovnin Volcano on Kunashir island has been found. Ethological barriers to mating between specimens of these populations appear to be absent both in the field and in laboratory. The embryos of the hybrid generation have a some mitosis disturbances. It caused the differentiated rate of survival the embryos death of different karyomorphs and the sterility of hybrid males. All its showed the isolation effect of a revealed chromosome rearrangements.

On Kunashir island a new species of the genus *Podisma* (describing as *P. tyatiensis* Sergeev et Bugrov) has been found. the karyotype of which have a short second arms in two pairs of autosomes and the X-chromosome ($2n\sigma=23$) which had not been found in other species of this genus.

All the above allows to suppose that in island grasshopper populations the speciation is accompanied by setting the chromosome isolation mechanisms.

08-113

GENOME MOBILIZATION IN TWO POPULATIONS OF *CHIRONOMUS RIPARIUS* MEIGEN 1804 (DIPTERA, CHIRONOMIDAE) FROM POLLUTED SEDIMENTS

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Two populations of *C. riparius* (from the Piedmont stations Moncalieri on the Po river and Santena on the Banna river) were studied from a cytogenetic point of view. Sediments of both stations had a concentration of some heavy metals (Cu, Cd, Pb, Cr, Fe) which was from two to three times higher than that observed in unpolluted sediments. Especially high was the body concentration of Cu, Fe and Pb in Moncalieri larvae and of Cd and Fe in Santena larvae. No pesticides or herbicides were present. Polytene chromosomes from salivary glands of IV instar larvae (prepupa stage) showed many structural rearrangements, i.e. paracentric heterozygous inversions, deletions, changes in appearance of heterochromatin, partial breakages, amplified as well as under-replicated regions, asynaptic zones, ectopic pairings and a different activity of Balbiani rings and NORs which often appeared in a heterozygous state. Such rearrangements were absent in standard lab populations. The IV chromosome (in which three Balbiani rings and a NOR are located) was the most involved in such rearrangements. However at any larval stage nearly absent were those morphological deformities which are generally considered as markers of a highly polluted environment.

The hypotheses are advanced that concentration of pollutants in the sediments of the two stations is sufficiently high to induce chromosome aberrations but not morphological deformities and that the observed genome mobilization could be considered as an early response to a stressful environment.

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08-112

KARYOTYPE EVOLUTION IN THE PARASITIC HYMENOPTERA V.E.Gokhman

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The results of chromosome study of more than 200 species of parasitic wasps have demonstrated that the plesiomorphous haploid chromosome number in the group (as well as in the whole Apocrita) is about 10-11. Numbers close to 10 occur in the majority of Ichneumonoidea, Cynipoidea, Diaprioidea, Scelionoidea, Ceraphronoidea and the families Encyrtidae and Eurytomidae from the Chalcidoidea. Evanioidea show higher n values (16). Lower chromosome numbers ($n = 3-7$) found in the Aphidiinae (Braconidae) and the most Chalcidoidea represent their synapomorphies.

Chromosomes of many parasitic wasps are predominantly two-armed and continuously decrease in size within a karyotype. A few ichneumonids (e.g. *Vulgichneumon saturatorius*) however, have highly asymmetric karyotypes, and acrocentric (or pseudo-acrocentric) chromosomes often predominate in wasps with higher haploid numbers.

Following mechanisms of change in chromosome number were detected: tandem fusions, robertsonian rearrangements, polyploidy, aneuploidy, numerical changes in B chromosomes. Among the structural changes, deletions and duplications (mainly of the constitutive heterochromatin), translocations and inversions were observed.

A few cases of population polymorphism have been detected in the parasitic Hymenoptera. C-banding polymorphism was found in *Dirophanes invisor* (Ichneumonidae). The data obtained for the ichneumonid *Tycherus bellicornis* suggest a translocation polymorphism resulting in aneuploidy. A unique B chromosome known in *Nasonia vitripennis* (Pteromalidae) converts diploid zygote into a haploid one.

It may be supposed at present that macroevolutionary changes of karyotype in parasitic wasps may involve virtually all types of rearrangements, and the most usual among them are: tandem fusions, aneuploidy, deletions and duplications of the constitutive heterochromatin, and translocations.

08-114

REVISION OF THE KARYOTYPES OF SPECIES BELONGING TO THE *DIPRION* GENUS (HYMENOPTERA : DIPRIONIDÆ)

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Earlier cytological investigations reported in Crozier's review (1975) characterize the Diprioninae with a range of chromosome numbers from 6 to 14 (with a very large majority of 7 as haploid number). In Maxwell (1958), *Diprion pini* and *D. similis* have a chromosome complement of 7 (haploid male) and 14 (diploid female) while Smith (1941, 1960) found *D. similis* haploid complement with 14 metacentric chromosomes. Their results are contradictory and difficult to explain because of the small size of the chromosomes.

Our present study demonstrates that both species *D. pini* and *D. similis* share the same karyotype : 14 chromosomes for haploid males and 28 for diploid females. The chromosomes are clearly telocentric with different lengths. Several banding methods are presented to characterize the heterochromatin localization. No chromosomal polymorphism was detected during the studies of four different populations of *D. pini*.

Our results disagree with the results previously published and reopen the question of karyotype evolution by doubling of the chromosome number.

08-115

THE LOWEST CHROMOSOME NUMBER OF TIMARCHA: VALIDATION OF T. AURICHALCEA (BECH.) A SPECIES WITH 18 CHROMOSOMES.

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The taxa belonging to the complex Timarcha goettingensis share a male meioformula of $9+X_{yp}$ ($2n=20$), where X_{yp} means a sex-"parachute" non-chiasmatic association, and a karyotype made of three large autosome pairs plus a large X-chromosome and six small autosome pairs plus the smallest Y-chromosome. A species of this complex, T. aurichalcea from Central Eastern Spain, has $8+XY$ ($2n=18$) and a karyotype differing from the former due to the lack of the 4th. acrocentric pair and the presence of a rather large Y-chromosome, which is held with the X-chromosome at metaphase I of meiosis by a chiasmatic aggregation (XY).

The Ag-NOR staining and fluorescent *in situ* hybridization, using a ribosomal DNA probe, demonstrates the cytogenetic origin of this species due to an event of centric fusion, changing also the sex-chromosome system from X_{yp} to XY.

08-117

GENETIC ANALYSIS OF WING DIMORPHISM AND RESPONSES TO WING-FORM SELECTION ON LIFE HISTORY TRAITS IN THE WHITEBACKED PLANTHOPPER, SOGATELLA FURCIFERA (HORVÁTH) (HEMIPTERA: DELPHACIDAE)

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The whitebacked planthopper, Sogatella furcifera (Horváth) exhibits wing dimorphism. The production of female macropters is most influenced by nymphal population density and is positively density-dependent. Bidirectional wing-form selection was imposed under low and high density conditions. Selection for increasing and decreasing the incidence of macroptery was most effective under antagonistic selection, i. e. selection for macroptery was most effective under low density conditions and selection for brachyptery was most effective under crowded conditions. Crossing experiments using the lines selected for macroptery and brachyptery suggest that the wing-form in S. furcifera is a threshold character under polygenic control and is determined by a threshold response to nymphal density. The realized heritability was 0.512 estimated from the lines selected for macroptery and 0.298 from the lines selected for brachyptery. The relationship between wing-form ratio and nymphal density (i.e. reaction norm) was parallel, which proved that there was no genotype-by-environment interaction between wing-form and density. There was a positive correlated response to selection on nymphal development period, but no consistent responses were detected on either fecundity or adult life span. A significant reduction in both forewing length and flight propensity of macropters was detected in response to selection for brachyptery, which suggests that flight propensity is positively correlated with the incidence of macropters.

08-116

KARYOTYPIC ANALYSIS OF THE GRASSHOPPER ANACRIDUM AEGYPTIUM (ORTHOPTERA, CATANTOPIDAE) BY CHROMOSOME BANDING TECHNIQUES

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During the past twenty years, chromosomal analysis has become notably refined due to the introduction of modern banding techniques which allow a detailed analysis of the chromosomal structure. Such techniques have successfully been employed in cytogenetics of higher vertebrates, man included.

Instead, the scantiness of data concerning G, R, and fluorochrome serial banding and restriction endonuclease digestion in invertebrates is thought to be the result of technical difficulties.

Nevertheless, attempts to correct this imbalance have recently been performed, mainly including chromosomal analysis by *in situ* endonuclease digestion of two species of the genus Tenebrio (Coleoptera: Tenebrionidae) and banding chromosomal analysis of some species of scarab beetles (Coleoptera: Scarabaeoidea).

With the exception of few examples of G banding, chromosomes of Orthoptera have been studied by means of Ag and C banding.

In this work we characterize the karyotypic constitution of the grasshopper Anacridium aegyptium (Orthoptera: Catantopidae) by employing i) conventional Giemsa staining and ii) differential Ag, C, G, R and DAPI banding.

Obtained results can be summarized as follows:

- 1) males of A. aegyptium show a karyotype which is mainly constituted by euchromatin and consist of 23 chromosomes with a chromosomal sex determining mechanism of the XO O/XX ♀ type.
- 2) All spermatogonial metaphase chromosomes exhibit Ag-positive signals, terminally located near the centromerical region.
- 3) Complementarity between G and R banding patterns was not observed
- 4) Centromerical areas appear DAPI-positive, thus suggesting the occurrence of A+T base-rich DNA in these chromosomal regions.

Two main implications originate from data achieved in our study:

- 1) whether or not silver proteins occurring near the centromerical region of all spermatogonial chromosomes are related to a real presence of rDNA; and
- 2) whether or not the lack of complementarity between G and R euchromatic serial bands may be due to the inhibition of the trypsin activity as suggested for other species of insects.

08-118

SOME OBSERVATIONS ON MATING FREQUENCIES IN DROSOPHILA

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The objective of this study was to test the generality of frequency dependent fitness in mating behavior of Drosophila, called minority effect and to investigate the effect of artificial olfactory cues and marking the flies by wing clipping on the frequency dependent mate choice.

In the mating experiments, using wild-type (oregon) and mutant (white-eyed, sepia, ebony) strains of Drosophila melanogaster mating success of mutant males did not increase, as their frequency decreased. On the contrary, mating success of mutant males, especially that of white-eyed mutant males decreased as their frequency decreased. In the experiments to test the hypothesis that females behave according to concentration of olfactory cues in their environment in mate selection, and chose the rare males, the olfactory cue hypothesis was not supported. Marking by wing clipping had an adverse effect on the mating success of male flies. Deviations from random mating due to marking were statistically significant in D. melanogaster whereas in D. funebris, wing clipping did not produce significant deviations from random mating.

The results of this study does not support the contention that rare male advantage probably, is an important factor maintaining genetic variation in natural populations of Drosophila.

08-119

THE TRADE-OFF BETWEEN MIGRATORY ABILITY AND MATE ATTRACTION IN THE SAND CRICKET, *GRYLLUS FIRMUS*

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Wing dimorphism is common in many insect species. While it has been shown that in females there is a trade-off between fecundity and wing morph, the short-winged (flightless) morph having a higher fecundity, there is little information on the possible cost of being capable of flight in the male sex. In orthoptera males attract females by calling, an activity that is energetically expensive. We hypothesized that the energetic costs of maintaining wing muscles decreases the energy available for calling, thereby reducing the number of females a long-winged male can attract. We tested this hypothesis using the sand cricket, *G. firmus*. The hypothesis was confirmed, short-winged males calling almost twice as long as long-winged males, and attracting 75% of the available females. These differences were accentuated when energy was made limiting. Full-sib analysis demonstrated that the above phenotypic trade-off has a genetic basis.

08-121

SEASONAL PHENOTYPIC PLASTICITY OF LIFE-HISTORY TRAITS BETWEEN DIAPAUSE AND NON-DIAPAUSE GENERATIONS IN A MULTIVOTINE BRUCHID, *KYTORHINUS SHARPPIANUS*

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In *Kytorhinus sharpianus*, the diapause-generation adults cannot lay enough eggs without feeding, but show significant longer adult longevity and preoviposition period than non-diapause-generation ones. The longer the period of chilling during diapause, the more conspicuous this tendency.

I conducted a experiment using split-brood design, in which full-sib offspring of a parent were reared under either diapause (short day + chilling) or non-diapause (long day) conditions. ANOVA indicated both significant effects of environment (condition during rearing) and brood on adult longevity. In the number of eggs deposited, only an environment effect was indicated. However, this population did not show significant environmentXbrood interactions in both traits. These results illustrated phenotypic plasticity and small variations in the slopes of reaction norms. I discussed that the phenology of its host plant, *Sophola flavescens* would be most important to have evolved the phenotypic variation between the diapause and non-diapause generations in *K. sharpianus*.

08-120

INFLUENCE OF *DROSOPHILA* MALE WING SIZE ON THE COURTSHIP SOUND. R. Tidon-Sklorz, F.M. Sene¹.

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In the genus *Drosophila*, the sound pattern produced by the vibration of the males' wings, during courtship, is important as an auditory stimulus for conspecific recognition and successful mating. This work tested if the availability of food in the larval stage, which is an environmental factor that affects the size of the wing, will affect the courtship sounds of *Drosophila mercatorum pararepleta*. Fifteen females of *D. mercatorum pararepleta* were placed in a bottle with 30 ml of culture medium for two hours, and transferred to another bottle with 10 ml of medium, where they were maintained for 72 hours. In the first bottle the larvae had abundant food during development; in the second bottle the larvae were poorly fed. Morphometric analysis confirmed that adults well fed during development had larger wings than those from the poorly-fed environment. Statistical analysis of some sound parameters (interpulse interval and fundamental frequency) showed that the difference in wing size affected the sound involved in female sexual stimulation; however, it did not affect the sound which mediates species recognition. This shows that the size of the wing when changed by environmental influence although changes some sounds produced by the males does not interfere in the courtship sound related to species recognition

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08-122

SHIFT IN PROPORTION BETWEEN PHENOTYPIC GROUPS IN *TRIBOLIUM* POPULATIONS AS THE REACTION TO ENVIRONMENT CONDITIONS

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Intrapopulation diversity has been reported in two species of flour beetles: *Tribolium castaneum* Hbst. and *Tribolium confusum* Duval. Two phenotypic groups existing simultaneously within populations differ in number of larval instars, rate of development and body weight. Recent studies show differences in energy distribution, rate and cost of reproduction and other traits what suggests different life strategies in the two phenotypic groups.

The objective of the paper is to verify hypothesis that the observed diversity improves plasticity of the species. It can be done by checking whether the proportion of the groups in population can shift as the response to changes in environment conditions.

Synchronized cultures have been run in different conditions (population density, degree of medium conditioning, disturbance by older individuals or presence of other species). Distinguishing between the two phenotypic groups has been made on the basis of development time and body weight of pupae.

Obtained results showed that increasing medium conditioning and the influence of other individuals stocked in higher numbers raises the proportion of the group with slower development within population.

08-123

GENETIC BACKGROUND AND ENVIRONMENTAL DETERMINATION OF EGG SIZE OF A SKIPPER, *PARNARA GUTTATA GUTTATA* (Lepidoptera: Hesperidae)

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The rice skipper *P. g. guttata* has three generations a year and the adult of the second generation migrates to southwest direction in September in Japan.

The egg size of the skipper varies among the three generations, i.e. small, small and large. The size of eggs seems to be an adaptive trait in relation to the leaf toughness of host grasses in each generation. This seasonal variation of the egg size is determined by the daylength during the larval period of mother. The egg size has also genetic background. The larger female lays larger eggs. Heritability of the egg size was relatively high, 0.78, and a genetic correlation was found between the female size and egg size. Selection experiment for both adult size and egg size was conducted for more than 15 generations, and the large and small egg size lines were obtained for both selections. However, the phenotypic plasticity of egg size to the daylength of the selected lines was not different from that of the nonselected line.

08-124

FORMING A DIAPAUSE CLINE BY SELECTION

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In crickets, three different patterns of egg development are observed, corresponding to different climatic conditions: stable diapause in the temperate region, opportunistic diapause in the subtropics and virtual absence of diapause in the tropics. Within some of the temperate univoltine species, there is a variation in the intensity of egg diapause in parallel to the latitudinal temperature gradient. To test the possibility that such macrogeographic as well as microgeographic clines in diapause were established by selection in the process of expansion of the distribution over various climatic areas, diapause incidence (the percentage of diapause eggs at LD 12:12 and 25°C) and intensity (the duration of the egg stage) were selected in a subtropical population of the ground cricket *Dianemobius fascipes* collected from Ishigaki Island (24°N) near the southern end of the Ryukyu Arc. Four lines were thus established to select for egg stages of about 3, 8, 15, and 22 weeks, respectively. Both the incidence and intensity of egg diapause varied in response to selection over many generations, and these selected lines showed a graded series of variation. In the long diapause lines, the diapause character changed from the subtropical opportunistic type into a more stable temperate type of *D. nigrofasciatus*. Thus a diapause cline was experimentally produced. However, the percentage diapause did not reach 100% even after 10-20 generations of selection in any diapause-selected line, suggesting the involvement of a polygenic system in controlling egg diapause in this ground cricket.

08-125

MEADOW GRASSHOPPER AND ALTITUDE - AN ADAPTIVE PUZZLE

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The morphometry and the colour polymorphism of *Chorthippus parallelus* (ZETTERSTEDT) were studied in 32 populations between altitudes of 300 m and 2.500 m above sea level in the Ore Mountains (field samples) and the Alps (collection Dr. Nadig, Switzerland). Despite of a considerable variation, the body size (hind femur) decreased with increasing altitude. Considering the distinctly shorter vegetation periods in the mountains, this coincides with laboratory results that smaller females reproduced earlier than greater ones due to energetical causes. With regard to the colour polymorphism of the populations, the percentage of darker morphs raised with increasing altitude. In field experiments significant differences up to 22 degrees between ambient temperature and body temperature of *Ch. parallelus*, depending on the amount of solar radiation energy, could be proved. But there is no clear correlation between the degree of melanism and the heat-up of individuals. The considerable heat-up of body temperature in dependence on insolation seems to be decisive for the completion of the life cycle and for reproduction of the species up to about 2.500 m above sea level at all.

08-126

THE SIMPLEST MEDIUM FOR REARING *DROSOPHILA MELANOGASTER*

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The author developed very simple and cheap rearing medium for fruit-fly *Drosophila melanogaster*. The medium consists of 30% of oat flour and 70% of water. The flour is put in the rearing tubes, then water is added. The tubes are closed with cotton plugs and are placed in the boiling water-bath for 45 minutes. After cooking, these tubes are dried a little in a drier (+80 - 100 C, 20-30 minutes), then they are cooled in the drier to the room temperature. This medium was compared with common medium, consisting of agar, yeasts, sugar and semolina. Several species of *Drosophila* were taken in the experiment, including wild-type and four mutant lines of *Drosophila melanogaster* (kindly given by Dr. I.K.Zakharov and Dr.B.F.Tchadov from the Institute of Cytology and Genetics, Novosibirsk, Russia). The mutant lines were reared on the medium, for at least 10 generations, and no deviations from the norm (as compared with common medium, mentioned above - it served as a control) in dimensions, development and fecundity were noticed. The proposed medium is free from expensive agar, sugar and yeasts (and it is not inoculated with yeasts). It is dense enough for flies. The residues of the medium are removed (during washing) not so easily, as in the case of agar-containing media, but the problem is solved by mechanical washers. Also the medium, composed of 5% of oat flour and 0,8-1% agar, is proposed.

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08-127

GENETICS OF COMPLEMENTARY SEX DETERMINATION: COUNTING SEX ALLELES IN *BRACON HEBETOR* (HYMENOPTERA: BRACONIDAE)

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We identified the sex alleles present in seven strains of *Bracon hebetor* that originated in various regions of the United States, and that had been cultured in the laboratory for various amounts of time. These alleles were then tested against *B. hebetor* populations available from commercial insectaries.

Within- and among-strain crosses to identify shared or divergent alleles were conducted using the production of diploid male offspring as a criterion indicating shared alleles in a cross. For some crosses, mutant phenotypic markers that segregate independently of the sex allele were available for identification of diploid males. For other crosses, however, such markers were not available, and the production of diploid males was confirmed using a combination of offspring survivorship data, sex ratio data, and karyotypes.

The survivorship of diploid male eggs was low when relative humidity was low, and higher when relative humidity was high. Also, mortality of diploid male eggs skewed the secondary sex ratio in predictable ways. We therefore compared the survivorship and secondary sex ratio of broods from crosses that were reared at either low or high levels of relative humidity. To compare secondary sex ratios with the fertilization rate, egg karyotypes were taken from complete broods. Finally, the production of diploid males was confirmed by karyotyping male pupae.

08-129

TRACKING THE EVOLUTIONARY HISTORY OF APOSEMATISM - EXPERIMENTS IN A NOVEL WORLD

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It is very hard to study the initial origin of aposematism, where unprofitable prey signal their unprofitability. After the long evolutionary history that predators have faced with aposematic prey items, it is difficult to unravel any inherited avoidance of common or conspicuous warning signals. Inherited effects do not only appear as immediate reluctance to attack aposematic prey, they may also enhance the avoidance learning or induce cautiousness of the attacks allowing the prey escape unharmed. To overcome the evolutionary history in the response of predators against warning coloration we created a novel world without colours using artificial symbols both in the background and as the signals. A series of experiments with great tits demonstrated that, initially, prey aggregation would have favoured the prey that signal their unpalatability. However, the clear advantage of clumping seems to disappear once birds have experience of similar warning signals. This context, representing Müllerian mimicry in a broad sense, is the situation where most of the unpalatable prey have evolved their warning signals. Previous experience enhances the learning by predators to avoid any new aposematic prey, even if the prey were solitary.

08-128

ALLOZYME SURVEY ON POPULATIONS OF *EUREMA HECABE* L. FROM JAPANESE MAIN AND SOUTHWEST ISLANDS (LEPIDOPTERA: PIERIDAE)

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The pierid butterfly, *Eurema hecabe* L., distribute widely in Japan and Southeast Asia. The populations in Okinawa and Ishigaki Islands showed some differences from the Main Is. population in its seasonal forms of the adult wing and in host plant preferences of larva.

We analyzed allozyme survey for populations from the Main Is. (Honshu, Shikoku and Kyushu), Okinawa Is. and Ishigaki Is. of Japan using a technique of the polyacrylamide gel electrophoresis. Obtained zymograms and allele frequencies of 15 loci were different among these populations, although they belong to a single subspecies *Eurema hecabe hecabe* in the present taxonomic status.

The allozyme data of the "Honshu-form" adults (which shown a wing pattern of Honshu type) collected in Okinawa Is. also different from the "Okinawa-form" butterflies, and they showed a close similarity to populations from main islands.

We will discuss the origin of "Honshu-form" butterflies on the basis of molecular, ecological and physiological aspects.

08-130

COLOR POLYMORPHISM IN THE HAWAIIAN HAPPY-FACE SPIDER, *THERIDION GRALLATOR* (ARANEAE: THERIDIIDAE): GENETIC MODIFICATION AND THE COUNTER ROLE OF SELECTION.

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The Hawaiian happy-face spider, *Theridion grallator*, an endemic to four islands in the Hawaiian archipelago, exhibits an exuberant color and pattern polymorphism involving both the carapace and opisthosoma (abdomen). Laboratory crosses, and broods from wild-mated females, demonstrate that the opisthosomal morphs on Maui (and probably on Moloka'i as well) act as if they are determined by alleles at one autosomal locus, although control by more than one linked locus cannot be eliminated. All morphs are found equally in males and females. In contrast, on Hawai'i Island, the 'yellow' and 'red front' morphs are controlled by a single allele, sex-limited in expression, such that females are 'yellow' and males 'red front'. 'Red blob' and 'red ring' form a similar pair of morphs, with 'red blob' confined to females, 'red ring' to males. In addition, there appear to be two unlinked loci involved in determining the color polymorphism on Hawai'i. The island-wide sex-limitation of 'yellow' and 'red front' suggests that the quantum shifts in the genetic control of the polymorphism among islands probably trace their origins to the founding event when *T. grallator* colonized Hawai'i from Maui.

Among color morphs, the relative frequency of morphs is remarkably constant among populations. $F_{ST}(\theta)$ values calculated using electrophoretic loci were much higher than those calculated using color morph alleles. This observation is consistent with selection operating to maintain the color alleles. We have suggested that bird predators are the most likely selective agent because of their ability to modify their feeding effort according to the frequency of a morph.

08-131

SPECIES PHYLOGENY OF THE BEE GENUS *EXONEURELLA* (HYMENOPTERA: APIDAE: ALLODAPINI) BASED ON MORPHOLOGICAL AND MOLECULAR DATA SET

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The members of the bee genus *Exoneurella* exhibit a range of social behavior from almost solitary to highly eusocial. *Exoneurella* forms the basal group for the rest of the endemic Australian allodapine bees, the *Exoneura* group. A cladistic analysis of the species relationships of the genus *Exoneurella* using morphological characters and sequenced data from cytochrome oxidase b of mtDNA was undertaken. Each data set analyzed separately gave slightly differing results. Morphological analysis using 41 adult, egg and larval characters resulted in one tree with minimal homoplasy and with the following species relationship: tridentata + (lawsoni + (eremophila + setosa)). Both strands of cyt b of the mtDNA were sequenced for the outgroup *Brevineura* and the *Exoneurella* species. Sequenced data analysis based on 383 bp of cyt b yielded one tree with higher homoplasy index than one based on morphological data set and with the following species relationship: tridentata + (setosa + (eremophila + lawsoni)). When morphological and molecular data were combined and analyzed, the resulting cladogram resembles the cladogram based on the morphological data set. The importance of historical patterns in looking at social evolution will be briefly discussed.

08-132

THE MOLECULAR PHYLOGENY OF MOSQUITOES IN ANOPHELINEAE AND CULICINAE (DIPTERA: CULICIDAE) BASED ON THE MITOCHONDRIAL GENE, CYTOCHROME C OXIDASE SUBUNIT II.

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With the polymerase chain reaction (PCR) and versatile primers that amplified the whole gene of cytochrome c subunit II (COII), we obtained 7 complete gene sequences represented three genera in Family Culicidae. These mosquitoes were *Anopheles sinensis*, *A. minimus*, *Aedes albopictus*, *A. togoi*, and *A. alsiaticus*, *Culex tritaeniorhynchus* and *C. annulus*. Together with other published sequences of four species, *A. gambiae*, *A. quadrimaculatus* and *A. aegypti*, and *C. quinquefasciatus*, provided a total of eleven mosquitoes sequences. This allowed a comparison of genes with a wide range of divergence. The comparison permitted an assessment of the phylogenetic relationships. In all cases, a single DNA fragment was obtained verified to a COII gene by Southern hybridization. These fragments were 685 bp in length. The deduced amino acid based on the nucleotides were 288 residues in length, beginning with conserved initiation methionine codon and terminating with an incomplete stop codon of T. Among these species nucleotide similarity ranged 81-99%, identity of deduced amino acid ranged 85-100%. The neighbor-joining trees based on the divergence of nucleotides and amino acids revealed the similar of branches.

08-133

MITOCHONDRIAL DNA EVOLUTION IN *DROSOPHILA IMMIGRANS* SPECIES GROUPS. C. Tsaur, C. T. Ting¹, S. Fang^{1,2}, F. J. Lin²

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The inferring phylogeny of *Drosophila nasuta* species subgroup of *D. immigrans* species group based on mtDNA as well as morphometric analysis has been compared in our previous studies. Despite the concordance lies between those two approaches, the mtDNA alone, however, shows some variations which including restriction site changes and size differences providing more information about the evolutionary process among these closely related species rather than morphological differentiations do. We report here the sequences of the mitochondrial cytochrome oxidase II gene of 25 species in the *D. immigrans* species group. This survey includes 5 members of the *D. hypocausta* subgroup, 3 of the *D. immigrans* subgroup, 14 of the *D. nasuta* subgroup, and 2 of the *D. quadrilineata* subgroup. The COII gene in this species group is 688 nucleotides in length as long as the *D. obscura* species group does, but 3 nucleotides longer than those of *D. melanogaster* and *D. yakuba*. The A+T content is high and show a strong transition bias as other *Drosophila* species do. The phylogenetic relationships in the species group are inferred using both neighbor-joining and maximum parsimony. The information provided by this sequence is in agreement with almost all other molecular and morphological data and suggested that the *D. nasuta* subgroup is a monophyletic group in *D. immigrans* species group. Furthermore, the size differences region of *D. immigrans* species will also be discussed.

08-134

EVOLUTION OF POLLEN FEEDING IN *HELICONIUS* BUTTERFLIES (LEPIDOPTERA: NYMPHALIDAE)H.W. KRENN¹, C.M. PENZ²¹Institute of Zoology, University of Vienna, Austria²Department of Zoology, University Texas Austin, USA

The neotropical *Heliconius* butterflies use their proboscis in addition to nectar feeding for the external collection of pollen and the subsequent uptake of the extracted amino acids (Gilbert LE 1972, Proc Nat Acad Sci USA 69: 1403-1407). Although this unique behavior has a central role in the biology of this group of Nymphalidae, the proboscis morphology and the pollen feeding behavior was not investigated in detail so far.

In order to reconstruct the evolution of this key innovation, the proboscis of 20 species of pollen feeding and non-pollen feeding Heliconiini was compared by means of light and scanning electron microscopy. The proboscis movements during flower visits and the pollen processing behavior were recorded in several species in the green house.

The morphological investigation did not show additional structures that exist exclusively on the proboscis of pollen feeders. However the comparison with non-pollen feeders revealed the reduction in the relative length of the proboscis tip, the modification of the length of sensilla, and a shift in the time pattern of proboscis movements. This distinct combination of features can be functionally interpreted in context with the collection and processing of pollen.

The evolution of pollen feeding and the implications for the systematical relationships in Heliconiini is discussed in reference to the features of the mouthparts and recent molecular data.

08-135

PHYLOGENIES DERIVED FROM NUCLEAR AND MITOCHONDRIAL GENES: COPING WITH PROBLEMS OF INCONGRUENCE, ALIGNMENT, AND INTRASPECIFIC VARIATION IN *ANOPHELES* MOSQUITOES

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Phylogenetic trees with conflicting topologies are frequently produced when phylogenies are constructed using multiple molecular data matrices. We present the results of a series of phylogenetic analyses using 380 bp of the nuclear *ITS 2* and 690 bp of the mitochondrial *COII* gene for 21 species in the *Anopheles* subgenus *Nyssorhyncus* and four outgroup species. When analyzed separately, each gene gave a slightly divergent phylogeny. We advance an incongruence metric for measuring disagreement between phylogenies.

Although alignment problems and intraspecific variation were not present in the *COII* gene, both were abundant in the *ITS 2*. Intraspecific variation within *ITS 2* was present in over 24% of the ingroup species. Alignment ambiguities due to multiple gap insertion prompted us to experiment with differential gap penalties and multiple alignments for *ITS 2*. Each alignment was then independently analyzed phylogenetically.

A phylogenetic hypothesis is advanced that includes both genes, but downweights *ITS 2* sites when they conflict with the *COII* phylogeny. This phylogeny greatly differs from a previous one based on morphological characters.

08-137

GENETIC STRUCTURE OF *OOPTERUS SOLEDADINUS* (COLEOPTERA, TRECHIDAE) SINCE ITS RECENT INTRODUCTION AT THE SUBANTARCTIC KERGUELEN ISLANDS.

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Oopterus soledadinus is a wingless predaceous beetle native from South Patagonia and Falkland Islands (51° 50' S). This species was accidentally introduced in 1912 at Port Couvreur on Kerguelen Islands (Southern Indian Ocean, 49° 20' S). Since its introduction, it only spread on littoral zone along the archipelago. Currently, it has colonized most of the eastern part of the Grande Terre (main island of the archipelago) with a maximal distance of 106 km from the introduction site.

We study genetic structure and morphometric features of three populations of *O. soledadinus* in order to describe their potential isolation. The reference population was sampled at the introduction site (Port Couvreur), the second one came from a littoral site 48 km away from Port Couvreur. Beetles from the third population were collected at the north side of Ile Haute (39 km away from Port Couvreur). The last two sites were selected owing to their distance from introduction site and to the kind of geographic isolation of the third one (sound). Eleven morphometric parameters were measured on both sexes of beetles from each population and several polymorphic allozyme loci were studied after carrying out a screening of proteins on the third population.

The colonization way of *O. soledadinus* is dependent on the interaction between its dispersal regime (flightlessness), its ecology (littoral species) and the occurrence of physical barriers. Did these features affect the genetic structure of this newly introduced species?

08-136

Cloning of microsatellites for parentage analysis and population studies in *Leptothorax* ants.

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A set of highly polymorphic microsatellite markers were developed for parentage analysis in multiple-queened colonies of *Leptothorax acervorum*.

When tested on a group of unrelated individuals they showed levels of heterozygosity ranging from 40-95%. To date one locus has yielded 44 alleles, ranging in size from 198-360 bp. The relationship between sequence composition and genetic variation among these diverse di-, tri- and tetranucleotide microsatellites will be discussed. These markers have been adapted for application on an ABI 373 automated sequencer, which allows for the multiplex amplification and electrophoresis of as many as 15 loci per individual. Our approach to this application will be discussed.

Finally, population genetic analysis of *L.acervorum* was carried out on a European-wide scale using the above markers. Results are discussed.

08-138

POLYMORPHISM IN PHOSPHOGLUCOMUTASE (PGM) AND HEXOKINASE (HK) IN *Apis mellifera* L. IN TURKEY

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We have investigated Phosphoglucumutase (PGM) and Hexokinase (HK) enzyme systems electrophoretically in *Apis mellifera* L. from samples collected in 16 localities over Turkey. Four alleles of PGM system were found. They were named as 100, 75, 63, and 45 according to their relative mobilities. This is the first report of four alleles found together in one study. Pgm-100 and Pgm-75 alleles were found in all localities. Gene frequencies of the most common allele (Pgm-75) ranged between 0.583-0.929. The Pgm-63 allele was found consistently in southern localities and the Pgm-45 was recorded only in one location from north-west of Turkey.

Three alleles of HK were found. They were named as 88, 100 and 110 according to their relative mobilities. HK-100 was the most common allele and found in all localities. The gene frequencies of this HK allele (HK-100) ranged between 0.679-0.992. HK-88 and HK-110 were the rare alleles and were found only in seven and six localities respectively.

08-139

POPULATION GENETIC STRUCTURE OF THE "JETHYS" COMPLEX OF *ENANTIA* (LEPIDOPTERA: PAPILIONOIDEA; PIERIDAE).

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Genetic structure and variation was studied in Mexican populations of three species that constitute the "jethys" complex of *Enantia* (Lepidoptera: Papilionoidea; Pieridae). These species have contrasting geographical distributions. *Enantia albania* and *E. mazai* have a wide distribution while *E. jethys* has a more restricted distribution. Twelve populations of *E. albania*, 9 of *E. mazai*, and 3 of *E. jethys* were studied for 10 enzyme loci. All three species have approximately the same number of alleles per locus (2) and high average heterozygosities (0.156-0.358). *E. albania* and *E. jethys* populations present allelic homogeneity due to high gene flow levels ($F_{ST}=0.096$ and $F_{ST}=0.044$ respectively). *E. mazai* populations have a greater genetic structuring ($F_{ST}=0.2325$). In this species, gene flow between population pairs (average $Nm=8.345$) is inversely related with the geographical distance between populations according to an isolation by distance model. This last result is consistent with the pattern expected for species in Hardy Weinberg equilibrium where gene flow is exclusively between adjacent populations.

08-140

POPULATION GENETICS OF EUROPEAN SALT MARSH CARABID BEETLES STUDIED BY ENZYME AND DISPERSAL POLYMORPHISM AT DIFFERENT SPATIAL SCALES

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We study population genetics of invertebrates in the framework of nature conservation. This case study reports on enzyme and dispersal polymorphism in the halobionth carabid beetles *Pogonus chaldeus* (Marsham) and *Dicheirotichus gustavii* (Crotch). The species are studied at different levels of spatial scale, in populations with varying size and isolation, in the remaining salt marshes in Belgium and reference sites at larger distance (France, U.K. and the Netherlands). Enzyme polymorphisms are studied by cellulose acetate gel electrophoresis. Dispersal polymorphism is studied by investigation of wing and flight muscle development.

Based on enzyme polymorphism, *D. gustavii* shows a larger genetic variation and, being the species with a relatively higher dispersal power, an expected smaller genetic differentiation between populations. Clear relationships between population or site characteristics and genetic structure are not observed, except for the special position taken by some small populations in both species. Concordant to these observations, the dispersal power of *P. chaldeus* in these small populations is larger, also leading to the conclusion that these populations are unstable and/or very young. They are however not always showing a lower genetic diversity as would be expected as a result of genetic drift in small populations.

Dispersal power in *P. chaldeus* declines with increasing age of salt marsh, probably due to a continuous emigration of winged individuals. The only exception relates to a population from Mont St. Michel (France) and is tentatively explained by the very large area of its salt marshes.

Age and size of salt marshes, although difficult to study independently, both appear to be important for the genetic structure of halobionth beetles. A maximal diversity in both parameters is therefore recommended as an optimal nature conservation strategy.

08-141

GENETIC STRUCTURE OF HOST ASSOCIATED POPULATIONS OF *YPONOMEUTA PADELLUS* (YPONOMEUTIDAE, LEPIDOPTERA): DO ALLOZYMES AND RAPDS TELL THE SAME STORY?

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Patterns of allozyme and Random Amplified Polymorphic DNA (RAPD) variation were used to estimate within and among population differentiation and levels of gene flow in *Crataegus* spp. and *Prunus cerasifera* infesting populations of the oligophagous small ermine moth, *Yponomeuta padellus*. A recently developed theory for the analysis of population structure with dominant RAPD markers was used to compare RAPD results with those obtained from allozyme analysis; both techniques were applied to exactly the same set of individuals. Allozyme frequencies differed significantly at 24 % of all polymorphic loci (19 in total) among all *Crataegus* populations, and at 31 % between the allopatric *P. cerasifera* populations. The number of loci with significantly different allozyme frequencies between populations on either hosts at two localities was 57 % and 50 %, respectively. The amount of gene flow, indicated by values of \hat{M} (i.e. the number of individuals exchanging per generation), between the sympatric *Crataegus* spp. and *P. cerasifera* populations was estimated at 1.9 and 2.2, respectively. These values were both considerably lower than the mean \hat{M} value of all pairwise comparisons of five allopatrically located *Y. padellus* populations on *Crataegus* spp. The RAPD data, however, did not corroborate these results. The estimates of population subdivision with RAPD markers, for example was much more pronounced than with allozymes and RAPD frequencies varied considerable among populations. We due these conflicting results to selection on the allozyme polymorphisms whereas the RAPDs are considered as neutral markers.

08-142

ALLOZYME VARIATION AND GENETIC STRUCTURE OF *CHIRONOMUS RIPARIUS* POPULATIONS FROM METAL CONTAMINATED AND NON CONTAMINATED SITES

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Saprotrophic arthropods are exposed to a large number of potentially toxic chemicals due to pollution of their natural habitat. Among these chemicals, contamination by heavy metals is an obvious example since they accumulate in soils and sediments. Because of their toxicity and persistence, contamination by heavy metals is a good example of how natural selection acts in nature. We therefore studied allozyme variation patterns to estimate within and among population differentiation of the midge *Chironomus riparius*. Populations from metal contaminated and non contaminated sites were sampled in the Dommel, a river system on the border of Belgium and the Netherlands with very high levels of copper and cadmium concentrations. Two indirect methods of estimating average gene flow levels were applied, i.e. Wright's *F*-statistics and the private allele method. Preliminary results showed that pollution by heavy metals do affect the population genetical structure of *C. riparius* populations. Nevertheless, it was also found that the geographical component was an even more important factor in shaping the genetical population structure. Since this can be the results of the fact that the genetic markers used in this study were not under the direct influence of natural selection or to loci linked by them, the number of genetic markers will be increased in the present future.

Section 9

Insect Molecular Biology
and Genetic Engineering

09-002

MODULATION AND TRANSDUCTION OF THE PHEROMONOTROPIC RESPONSE TO PBAN
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Pheromone production by many moth species is regulated by the timely release of pheromone biosynthesis activating neuropeptide (PBAN) which signals the induction of pheromone production by female moths. Our *in vitro* studies¹ on *Helicoverpa armigera* have resulted in the identification of the target cells for neuropeptide action. These cells are situated in the intersegmental tissue between the 8th and 9th abdominal segments of female moths. No pheromonotropic activity was observed in incubations of the 8th segment alone. The neurotransmitter, octopamine and its analogs, tyramine and clonidine, were observed to inhibit and modulate the pheromonotropic action due to PBAN. The biochemical second messenger system during both pheromonotropic (PBAN) and pheromonostatic (adrenergic agonists) activities was studied. Several second messengers (cAMP, calcium and phosphatidylinositols) were implicated as transducers of PBAN action². The ionophores, ionomycin and thapsigargin, stimulated both pheromone biosynthesis and intracellular cAMP production. Both activities (pheromone and intracellular cAMP production) were inhibited by calcium-calmodulin inhibitors (W12 & W7). The involvement of G-proteins was implicated by the calcium-dependent stimulatory action of NaF (1mM), which, like PBAN, stimulated both pheromone biosynthesis and intracellular cAMP production. This stimulatory action was significantly inhibited by adrenergic agonists and was reversed in the presence of pertussis toxin thus implicating G_i in the modulation of pheromonotropic activity. From our studies we conclude that a negative regulation of pheromone biosynthesis occurs at the membrane receptor level by the interaction of an adrenergic receptor with the PBAN receptor.

References:
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2. Soroker, V. & Rafaeli, A. (1995) Insect Biochem. Molec. Biol. 25: 1-9.

09-001

SPATIOTEMPORAL ASPECTS OF CALCIUM SIGNALLING

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Cells employ both external and internal sources to generate Ca²⁺ signals which are often organized in complex spatial and temporal patterns. Many stimuli use inositol 1,4,5-trisphosphate (InsP₃) to control both the release of internal Ca²⁺ and the influx of external calcium. Opening of the InsP₃ receptors on the endoplasmic reticulum is controlled by both InsP₃ and Ca²⁺ and this dual regulation forms the basis of a model to explain both Ca²⁺ spiking and Ca²⁺ waves. Continuation of this oscillatory cycle is often dependent upon the influx of external Ca²⁺. Particular attention will be focused on the processes of capacitative Ca²⁺ entry which is regulated indirectly by InsP₃ through its ability to empty the Ca²⁺ stores. Recent work in *Xenopus* oocytes and *Drosophila* photoreceptors have characterized both the kinetics of this entry process and its sensitivity to Ca²⁺. The results are discussed in relation to a conformational coupling mechanism whereby the head of the InsP₃ receptor is responsible for transferring information from the E.R. to the plasma membrane.

Calcium entering from the outside is then taken up by the internal stores from which it is released periodically as Ca²⁺ spikes. The initiation processes seem to depend upon the integration of unitary Ca²⁺ events which have been referred to as bumps, sparks and puffs. Co-ordination of these unitary events, often achieved through the regenerative processes of Ca²⁺-induced Ca²⁺ release (CICR), results in the global Ca²⁺ signals responsible for cell activation.

09-003

ROLE OF CALCIUM AND PHOSPHOINOSITIDES IN THE REGULATION OF JUVENILE HORMONE BIOSYNTHESIS IN COCKROACHES

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Juvenile hormone (JH) biosynthesis is regulated by peptidergic inputs. Depending upon the species and developmental stage, these signals may be either inhibitory or stimulatory (allatostatins and allatotropins). Signal transduction within corpus allatum cells occurs by way of known second messengers, including the cyclic nucleotides, calcium and the phosphoinositides. In the cockroach, *Diploptera punctata*, allatostatic signals appear to be the principal regulators of JH production. Receptors for allatostatins have been partially characterized from brain and corpus allatum of *D. punctata*. These receptors probably differ between tissues and probably occur as distinct sub-types, which may be a function of the large number of allatostatins and their wide range of actions.

Modulation of intracellular calcium levels by drugs (ionophores, calcium chelators and releasing agents, calcium channel blockers) or depolarizing agents, including current injection, dramatically alters JH biosynthesis, as do agents that activate the phosphoinositide pathway. Allatostatins also appear to directly affect calcium flux in corpus allatum cells. Modulation of intracellular cAMP levels is associated with changes in JH production, with increases in JH biosynthesis occurring as a consequence of the stimulation of the adenylate cyclase system in some species (e.g. locusts) but with an inhibition of JH biosynthesis in cockroaches. The effects of second messengers differs between species, probably as a consequence of the differences in the peptide signals that regulate JH biosynthesis and in the intrinsic state of the corpora allata (either switched on or switched off, in the absence of external signals).

09-004

NITRIC OXIDE: A NEW SIGNALLING MOLECULE IN THE INSECT CNS

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Nitric oxide (NO) is now recognised as a neuronal signalling molecule in the mammalian brain. NO is synthesised in nitrergic neurons by a Ca²⁺-calmodulin activated nitric oxide synthase (NOS) which catalyses the conversion of L-arginine and molecular oxygen to NO and citrulline. NO activates the enzyme soluble guanylyl cyclase (sGC) producing the second messenger cGMP.

We have demonstrated the existence of the NO cGMP signalling pathway in the insect CNS. The optic lobes show extensive and locally intense staining using the NADPH diaphorase technique. NOS is also associated with local interneurons in the olfactory lobes where NOS staining in fibres defines the glomerular organisation. An association between the NO and learning and memory is suggested by entirely new and unexpected structures within the mushroom bodies. The α and β lobes of the mushroom bodies contain tubular structures defined by strong circumferential NOS staining. These are the first sub-compartmental structures to be described in the mushroom bodies, parts of the insect brain associated with learning and memory. Biochemical and molecular studies indicate the presence in the insect CNS of the two principal enzymatic components of the signalling pathway, namely NOS and sGC, with considerable sequence identity with their mammalian counterparts. This talk will review our progress towards understanding the molecular biology, cellular localisation and functional role of this signalling pathway in the insect brain. Grant BBSRC GR/J33234.

09-005

INTERCELLULAR SIGNALLING AND THE REGULATION OF CELL DIVISION IN THE MALPIGHIAN TUBULES OF *Drosophila melanogaster*

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The excretory system of the fly consists of four Malpighian tubules, which arise from the embryonic hindgut by eversion of primordial cells. Cell proliferation in each of the four primordia is completed during a short period of embryogenesis. The pattern of cell proliferation is precise and reproducible. We have identified components of the regulatory network governing cell division and shown a requirement for the activity of two signalling pathways: one mediated by *wingless* and the other by *spitz*, through the activation of the *Drosophila* EGF receptor (DER). In addition, the activity of a single cell in each tubule, the tip cell, is required. The phenotypes of the Malpighian tubules in embryos mutant for elements of the signalling pathways and those in which the normal specification and differentiation of tip cells is perturbed, indicate that the combination of triggers that stimulate cells to divide change during tubule development. The relationship between these regulatory factors and the passage of responding cells through cell cycle checkpoints is currently being investigated.

09-006

BETWEEN PEPTIDE HORMONE AND STEROID HORMONE: THE PTTH TRANSDUCTORY CASCADE.

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Ecdysteroids coordinate molting and metamorphosis of insects. In pre-adult Lepidoptera, the prothoracic glands produce ecdysteroids, under the acute control of the brain neuropeptide prothoracicotropic hormone (PTTH). In the 1980s, *in vitro* studies, using the model insect *Manduca sexta*, indicated that PTTH stimulates rapid intracellular increases of Ca²⁺ and cAMP in prothoracic glands. More recent studies demonstrate that changes in these intracellular second messengers are followed rapidly by prothoracic gland-specific changes in the phosphorylation state of at least 1 protein (the ribosomal protein S6) and the rapid synthesis and accumulation of 3 to 6 proteins (including β tubulin and the heat shock protein, hsc 70). This suite of intracellular events appears to be required to support the rapid increase in ecdysteroid synthesis that normally accompanies PTTH stimulated, possibly by controlling the rate of movement of ecdysteroid precursors among intracellular compartments. Some of these molecules may also play a long term role in modulating the growth (size) of prothoracic gland cells, and by doing so, effectively set upper and lower limits on ecdysteroid production by PTTH-stimulated and unstimulated (resting or basal) glands, respectively.

09-007

THE OVARIAN INSULIN RECEPTOR - A POTENTIAL ROLE IN THE REPRODUCING MOSQUITO

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When the female mosquito, *Aedes aegypti*, takes a blood meal, a cascade of hormonal events is initiated which maintains vitellogenesis and oocyte maturation. The major hormone(s) with gonadotropic action, the ovarian ecdysteroidogenic hormone (OEH), induces ovarian ecdysteroidogenesis directly and vitellogenesis indirectly. In some insects, ecdysteroid secretion has been linked to the presence of insulin-like material and we have found that vertebrate insulin stimulates this process in mosquito ovaries *in vitro*.

In vivo, ovaries increase their protein synthesis rate several-fold after activation by a blood meal, making protein synthesis a simple indicator for the activation state of the ovary. In an *in vitro* protein synthesis assay we demonstrated that insulin can induce a two to three fold increase in previtellogenic ovaries. Based on this lead, we have proposed that insulin receptors transduce hormonal signals for ecdysteroidogenesis in the mosquito ovary. By sequence homology, we have designed PCR-primers to synthesize fragments coding for conserved subdomains of tyrosine kinases. One such fragment was used to clone cDNAs from ovaries. An open reading frame (mosquito insulin receptor, MIR) shows the typical characteristics of a receptor tyrosine kinase: a cysteine-rich extracellular domain, a processing site for precursor cleavage into α - β -subunits, a transmembrane domain in the β -subunit followed by consensus sequences for tyrosine kinases. Expression analysis of mRNA indicated a highly specific accumulation of MIR-mRNA in ovaries. *In situ*-hybridization demonstrated expression in the nurse and follicle cells before and after a blood meal. Towards the end of the egg maturation process, a decrease of MIR-mRNA was observed along with the degeneration of follicle cells. Immunocytochemical detection of MIR demonstrated a colocalization with MIR-mRNA.

Our observations support the conclusion that the mosquito insulin/insulin receptor homologs play a crucial role during egg maturation.

09-008

INDEPENDENCE OF HOST FACTORS MAY EXPLAIN THE WIDESPREAD DISTRIBUTION AND HORIZONTAL TRANSFERS OF *MARINER* TRANSPOSONS

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Mariner transposons have been shown to be extremely widespread in animals. In addition to the many hundreds of representatives identified in diverse insects, they have been found in mites, flatworms, hydras, and primates. They can be organized into five major subfamilies, and at least twelve additional smaller subfamilies. *Mariners* are related to the *Tc1* family of transposons also found widely in animals, and more distantly to a diverse superfamily of transposons such as IS630, IS3, Tn10 and Mu bacteriophage, as well as the integrases of retroviruses. These transposases share a conserved D,D35E catalytic domain, and catalyse transposition by a cut-and-paste mechanism.

The evolutionary relationships of *mariner* transposons clearly demonstrate that particular elements have been transferred horizontally between hosts, both recently and anciently. Sometimes these hosts are in different orders of insects or even phyla of animals. These observations suggest that they can function independently of host factors. We have developed an in vitro transposition assay for an irritans subfamily *mariner* from the horn fly, *Haematobia irritans*, called *Himar1*, in which transposition of a marked element between plasmid substrates is catalysed by purified transposase protein. These results are similar to those obtained for retroviral integrase, and may apply to most members of the superfamily, indicating that host factors are not required for transposition.

Several attempts to obtain activity of *Himar1* in *Drosophila melanogaster* as a test system have failed, but we continue to test this construct as well as other *mariners* for activity in this fly in preparation for development as genetic tools for other insects. Given their wide distribution and independence from host factors, various *mariners* may prove to be useful genetic tools in other animals too. It remains to be seen, however, whether organisms have "defences" against transposable elements that might limit the host range of particular *mariners*.

09-010

THE USE OF MINOS TRANSPOSONS FOR GERM LINE TRANSFORMATION IN INSECTS

B. Savakis (Crete-Greece)

ABSTRACT NOT RECEIVED

09-009

HAT TRANSPOSABLE ELEMENTS AS GENE VECTORS IN INSECTS.D. A. O'Brochta and P. W. Atkinson¹

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HAT transposable elements are members of a family of transposable elements that share a number of structural and functional characteristics. They are short, have conserved inverted terminal repeats, encode transposases containing common domains, and leave similar footprints at empty sites following excision from DNA. Members of the *hAT* element family isolated and characterized from plants have been found to be capable of accurate transposition in plant species distantly related to their original host species. Insect *hAT* elements are widespread and are also capable of accurate transposition in non-host species. The *hobo* element of *Drosophila melanogaster* can transpose when introduced into tephritids, muscids and calliphorids. Likewise, the *Hermes* element of *Musca domestica* can transpose in these and other families, including culicids and noctuids. Significantly, the *Hermes* element has been used to achieve genetic transformation of a non-host species, *D. melanogaster*, suggesting that this and other *hAT* elements may be harnessed as gene transfer vectors in a wide range of insect species. We will present data showing the structure and mobility properties of several insect *hAT* elements. In addition we will discuss how transposable element mobility assays can be employed to determine the mobility properties of members of the *mariner* element family.

09-011

THE PRESENCE OF RETROVIRUSES IN INSECTS AND THEIR POTENTIAL USE TO DEVELOP RETROVIRAL VECTORS FOR TRANSGENESIS

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Retroviruses were considered to be restricted to vertebrates. However the genomes of many eukaryotes including insects contain transposable elements structurally related to proviruses of retroviruses, called LTR-retrotransposons. They contain LTRs (Long Terminal Repeats), but have only two ORFs corresponding to the genes *gag* and *pol*. They are not infectious because they are devoid of a third ORF coding for Envelope products that are responsible for the infective properties of retroviruses. Some insects contain elements like gypsy in *Drosophila melanogaster* that are much more similar to typical retroviruses than to LTR-retrotransposons. Gypsy contains three ORFs coding for putative polypeptides showing similarities to *gag*, *pol* and *env*.

We have shown that gypsy is an infectious retrovirus, the first retrovirus characterized so far in invertebrates. It can be transmitted from strains in which it transposes actively to strains devoid of functional elements, by egg plasm transplantation or by growing "empty" larvae in the presence of homogenized pupae of a stock containing transposing elements.

Multiplication of gypsy in the germline is under the control of a polymorphic locus that we called *flamenco* (*flam*) which maps at 20A1-3. This gene has a maternal effect on gypsy mobilisation: transposition occurs only in the progeny of females homozygous for permissive alleles of *flamenco*. We have shown that the transcripts of gypsy in *flam/flam* females accumulate in the somatic follicle cells of the ovaries. We think that particles are produced in these cells and that they infect the oocyte. Transposition observed in the next generation would correspond to integration of gypsy proviruses in flies derived from infected oocytes. We think that gypsy can be used to develop vectors in the same way as mammalian retroviral vectors. They might be used for transgenesis not only in *Drosophila melanogaster* but also in other insects.

09-012

INTEGRATION AND EXPRESSION OF PANTROPIC RETROVIRUSES INTO CELLS OF THE MALARIA VECTOR, *ANOPHELES GAMBIAE* (Giles) (DIPTERA: CULICIDAE)

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The lack of efficient techniques for stable genetic transformation of medically-important insects, such as anopheline mosquitoes, is an impediment to progress in developing novel disease control strategies. Currently available techniques for foreign gene expression in insects lack the benefit of stable inheritance conferred by integration. To overcome this problem, a new class of pantropic retroviral vectors has been developed in which the amphotropic envelope of the murine leukemia virus has been replaced by the G glycoprotein of vesicular stomatitis virus. The broadened host range of these viral particles allows successful entry, integration, and expression of heterologous genes in cultured cells of *Anopheles gambiae*, the principle mosquito vector responsible for the transmission of over 100 million cases of malaria each year. Mosquito cells in culture infected with a pantropic vector expressing hygromycin phosphotransferase from the *Drosophila melanogaster* hsp70 promoter were resistant to the antibiotic Hygromycin B. Integrated provirus was detected in infected mosquito cell clones grown in selective media. Mosquito-viral DNA junction fragments were isolated and sequenced providing evidence that the integration is viral-mediated. Thus, pantropic retroviral vectors hold promise as a transformation system for mosquitoes *in vivo*.

09-013

RETROTRANSPOSABLE ELEMENTS THAT SPECIFICALLY INSERT INTO THE 28S RIBOSOMAL RNA GENES: THEIR DISTRIBUTION IN INSECTS AND USE FOR GENETIC TRANSFORMATION

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R1 and R2 are two different non-long-terminal repeat (non-LTR) retrotransposable elements which insert in the 28S ribosomal RNA genes. The insertion specificity of these elements has enabled rapid progress in studies of their distribution, evolution and retrotransposition mechanism. R1 and R2 have been found in all insect lineages and in all classes of Arthropods, where together they occupy from a few percent to over half of the total number of rDNA units. Their ability to be maintained within each lineage is predominantly a result of their vertical descent through the germline. The single ORF of the *Bombyx mori* R2 element encodes both an endonuclease specific for the 28S target site and a reverse transcriptase activity. The fundamental step in the R2 integration reaction is the cleavage of one strand of the 28S target site by this endonuclease, and the use of the 3' hydroxyl group exposed by the nick to prime reverse transcription of the R2 transcript. This mechanism, termed target-primed reverse transcription or TPRT, is therefore completely unlike the mechanism used by LTR-retrotransposable elements and retroviruses. Recently we have begun to utilize our understanding of the TPRT reaction to develop R2 into a transformation vector. When the R2 protein and RNA molecules containing the 250 nucleotide 3' untranslated region of a *B. mori* R2 element are injected into *Drosophila melanogaster* 1-2 hour embryos, integrated *B. mori* R2 elements are detected in the 28S genes of some adult survivors (somatic integrations) as well as in the offspring of these animals (germ-line transformation). Because entirely Lepidopteran components can be successfully used with a Dipteran, we are hopeful that this approach will work in many insects.

09-014

IKIRARA, A POTENTIALLY ACTIVE *ANOPHELES GAMBIAE* TRANSPOSON WITH SIMILARITIES TO *hAT* AND *Tc1*-LIKE ELEMENTS.

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A novel DNA to DNA transposon *Ikirara* was found in the genome of the malaria vector mosquito, *Anopheles gambiae*. Related sequences were found in other members of the species complex, but not in *A. funestus* or *A. stephensi*. *Ikirara-1*, the first isolated family member, was found between two of the tandem vitellogenin (Vg) genes. Because this particular insertion was found only in one of nine tested *A. gambiae* s.s. strains, and because its 216 bp inverted terminal repeats are 100% identical, it is possible that transposition to the Vg locus was recent. The strain with the *Ikirara-1* insertion also appears to have greater variability in the genomic locations of its other *Ikirara* elements than strains lacking the insertion, but from the same geographic location and carrying the same chromosomal inversions.

DNA sequence derived from 5 different *Ikirara* elements showed similarities to two transposon superfamilies, *hAT* and *Tc1*-like elements found in a variety of animals and plants. The ends of the inverted repeats and the insertion site TA duplication are most similar to *Tc1*-like elements, while sequences internal to two of the elements, *Ikirara-29* and -25, show similarity to the conserved DNA-binding and carboxy termini of *hAT* transposases.

An excision assay based on *Ikirara-1* was developed to determine whether any genomic *Ikirara* elements are currently active. After passage of the excision tester plasmid through *A. gambiae* tissue culture cells, several putative excisions were recovered and sequenced. They showed complete transposon loss except for a short "signature" sequence.

09-015

SPECIES, POPULATION AND CHROMOSOME DISTRIBUTION OF A NOVEL FAMILY OF NON-LTR RETROTRANSPOSONS ISOLATED FROM THE SANDFLY *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE)

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We have isolated members of a unique family (Rlppapa) of non-LTR retrotransposons (RTPs) which were found to have an atypically long 3' untranslated region (UTR) and an unusual repetitive "tail" sequence downstream of the polyadenylation signal. Examples of Rlppapa were isolated from *Phlebotomus (Phlebotomus) papatasi* by screening a genomic library with probes of homologous reverse transcriptase (RT) fragments cloned from PCR products. Contiguous Rlppapa restriction fragments from each genomic clone were sequenced from the RT gene downstream through the tail and into the DNA flanking each RTP. Using Southern and dot-blot hybridization analyses, Rlppapa sequences could be detected only in the genomic DNA of populations of *P. papatasi* and the related *Phlebotomus (Phlebotomus) duboscqi* but not of sympatric sandflies classified in other subgenera, suggesting vertical transmission of these RTPs. PCR was used to assess differences in chromosomal site occupancy (by employing reverse primers specific for each of the cloned flanking regions) and in the structure of different members of the Rlppapa family (by sequencing the variable 3' UTR). Phylogenetic analysis demonstrated the presence of 5 distinct subfamilies of Rlppapa, each of which was found in widely-separated geographical populations of *P. papatasi*. FISH, Southern hybridization and sequencing indicated a varied chromosomal distribution, with Rlppapa RTPs being detected in coding as well as non-coding regions. This observed variation suggested (recent) transpositional activity. This project is supported by a grant from the CEC/EU.

09-016

CHARACTERISATION OF A PUTATIVE FULL LENGTH MARINER ELEMENT IN THE MEDFLY *CERATITIS CAPITATA*.

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The genome of the Mediterranean fruitfly, *C. capitata*, has previously been shown to contain several subfamilies of *mariner* elements (Robertson & MacLeod. Insect Molecular Biology 2: 125, 1993). However, in every case the elements were clearly non-functional due to multiple stop codons, deletions, insertions and frameshifts. Despite this discouraging background we were determined to identify a putative full length mariner element.

Mariner elements were amplified using primers complementary to the Inverted Terminal Repeat sequence of the deleted *mariner*-like element found in the *Adh1* intron of *C. capitata* (see accompanying poster of Gomulski et al.). A fragment of around 1300 bp was amplified from total genomic DNA. A number of clones were sequenced, the majority of which contained deletions and frameshifts. One clone, however, was found to possess an ORF of 339 amino acids with ~ 60% similarity to that of the *mos* element of *Drosophila mauritiana*.

The phylogenetic relationships between the *mariner* elements of *C. capitata* and other species were investigated. Those isolated in the present study are representatives of Robertson's mellifera subfamily.

09-018

GENE EXPRESSION IN THE INSECT FAT BODY

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In insects, the fat body is the center of intermediate metabolism and is a powerful secretory organ producing hemolymph proteins. Elucidation of the molecular mechanisms underlying expression of fat body genes is of great importance to the effort toward utilization of molecular genetics in developing novel control strategies, particularly for vector insects. Expression of most fat body genes is controlled in a sex-, tissue-, and stage-specific manner. Transcription factors involved in these gene-regulatory pathways act synergistically to activate transcription of target genes. For the mosquito fat body, we have identified and cloned the cDNAs of transcription factors linked to the hormonal gene-regulatory pathway (ecdysteroid receptor and Ultraspiracle homolog) and the tissue-specific gene-regulatory pathway (hepatocyte nuclear factor 4 homolog, AaHNF4). Three isoforms of the AaHNF4 have been cloned and analyzed. Finding high expression of HNF4, a key transcription factor in the liver, in the mosquito fat body affirms the conservation of regulatory mechanisms for tissue-specific transcription. The regulatory regions of two fat body-specific mosquito genes are being analyzed: vitellogenic carboxypeptidase, a yolk protein precursor gene, expressed exclusively in the vitellogenic fat body, and lysosomal aspartic protease, expression of which is enhanced in this tissue. Regulatory regions of both these genes contain similar putative fat body enhancer elements which are homologous to the *Drosophila* yolk protein gene enhancer required for sex- and tissue-specific expression and contained Dsx and bZIP binding sites.

09-017

GENE EXPRESSION IN THE SALIVARY GLANDS OF THE MOSQUITO *Aedes Aegypti* (L) (DIPTERA: CULICIDAE)

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The salivary glands of the vector mosquito, *Aedes aegypti*, express two classes of genes whose products are involved in feeding. One class of genes is expressed in both males and females, and the products appear to facilitate the ability of the mosquito to feed on sugars. The second class is expressed preferentially in females and its products are involved in hematophagy. The promoters of select salivary gland-specific genes have been evaluated for function in a number of assay systems including expression in mosquito cultured cells isolated salivary glands in culture, a transcription system *in vitro*, and by transposon-mediated transformation into the mosquito and the fruitfly *Drosophila melanogaster*. A putative promoter fragment from the *Maltase-like 1* gene functions in most of the systems tested while promoters of female-specific genes (*D7* and *Apyrase*) are more restricted in their ability to drive expression of reporter genes. Tissue-specific gene promoters can be used to target the expression of coding sequences that could interfere with parasite development and propagation.

09-019

IDENTIFICATION OF THE TARGET FOR SEX-PEPTIDE IN *DROSOPHILA MELANOGASTER*

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Sex-peptide (SP), a male accessory gland peptide of *D. melanogaster*, is transferred to the female during copulation, and elicits reduction of receptivity and stimulation of ovulation/oviposition. Little is known about the primary target tissue(s) of SP in the females. We have attempted to identify the target(s) of SP with a novel strategy involving GAL4-UAS ectopic expression system. We constructed a membrane-bound form of SP, and expressed in a variety of tissues using randomly selected GAL4 enhancer trap lines. 33 out of 204 GAL4 lines showed both behavioral change and stimulated ovulation. The remaining 171 lines did not show any phenotypes, suggesting that there is only one target site for the two reaction pathways. Expression pattern analysis with tissue sections as well as Western blotting of head, thoracic, and abdominal extracts demonstrated that most of the positive lines express GAL4 in their heads. In the head, GAL4 was preferentially expressed in the antennal lobes, antennal nerves, and/or mushroom bodies, suggesting that SP could elicit behavioral changes and ovulation through interacting with the olfactory signal processing pathway.

09-020

THE ACETYLCHOLINESTERASE GENE OF *ANOPHELES ALBIMANUS*¹L. M.C. Hall, ¹C. A. Edwards and ²C.A. Malcolm¹Department of Medical Microbiology, London Hospital Medical College, University of London UK² School of Biological Sciences, Queen Mary and Westfield College, University of London UK

The acetylcholinesterase gene from insecticide susceptible *Anopheles albimanus* has been sequenced in full. The encoded protein sequence is very similar to that from *Anopheles stephensi*, with only 4% amino acid differences in the mature protein sequence (excluding the signal sequence and the excised C-terminal peptide). The DNA sequence encoding the mature protein differs at 20% of bases, but intron sequence have no significant homology between the species. The position of introns is conserved between the species, except that *An. albimanus* lacks the third intron of *An. stephensi*, which is also absent in *Drosophila melanogaster*. Intron lengths are very short.

The DNA sequence has also been determined from individual insects from an insecticide resistant population of *An. albimanus* (FEST). This was performed by direct sequencing of PCR amplified fragments. An almost complete sequence from one individual shows no nucleotide changes within coding sequences, and only three changes within intron sequences. This was further confirmed by determining the sequence encoding the lining of the active site in individual insects in which acetylcholinesterase assays from the heads had demonstrated homozygous resistance. The sequences known to be involved in resistance in *Drosophila melanogaster* and housefly were included in this region. Hence the mechanism of resistance in this population remains unclear.

09-021

CHARACTERISATION OF CHYMOTRYPSIN GENE STRUCTURE, EXPRESSION, AND ORGANISATION IN THE MALARIA MOSQUITO *ANOPHELES GAMBIAE*G.J. Lycett, H-M. Muller¹, A. Crisanti¹, J.M. CramptonLiverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK - ¹ Istituto di Parassitologia, Università degli Studi di Roma, La Sapienza, 00185 Roma 1, Italy.

The aim of this research is the characterisation of serine proteases in the malaria mosquito, *An. gambiae*. These enzymes are known to be agents of blood meal digestion and there is also evidence of their possible role in activating key enzymes in malaria parasite development in the mosquito host. We have identified and cloned a family of chymotrypsin genes from the KWA strain of *An. gambiae*. This family is clustered and contains 4 genes from hybridization studies. Two partially sequenced genes share 99% homology with the chymotrypsin genes (anchy1 and anchy2) previously isolated from the Suakoko strain of *An. gambiae*. We have completely sequenced a third gene (anchy3) that has 74% and 76% homology with the anchy1 and 2, respectively. Evidence from Southern blot hybridisation studies suggests that anchy3 is also present in the Suakoko strain, but has not yet been isolated from this source.

This analysis will eventually allow a direct comparison of the structure and function of the protein coding regions and possible regulatory regions of these very important protease genes.

09-022

MOSQUITO FERRITIN: TRANSCRIPTIONAL OR TRANSLATIONAL REGULATION?

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In vertebrates, iron plays an essential role in many cellular processes, and perturbations of iron homeostasis often result in detrimental consequences. We started a study of iron metabolism in insects with the idea that knowledge obtained from this study could provide a means to control disease vectors. We initiated our study by examining ferritin (Fer), a well-known iron-storage protein in vertebrates. The expression of vertebrate *fer* is regulated mostly at the translational level. Previously, we have reported the isolation and sequence of a cDNA clone for a Fer subunit in the yellow fever mosquito *Aedes aegypti*. We now present the regulation of the *A. aegypti fer* and its homologue in the forest day mosquito *A. albopictus*.

In *A. albopictus* cultured cells, a moderate induction of Fer was seen at 4 hrs post-treatment with iron, and the accumulation of Fer continued until 24 hrs post-treatment. When *A. albopictus* cells or *A. aegypti* mosquitoes were exposed to actinomycin D prior to iron treatment, the level of *fer* mRNA was reduced significantly. This result suggested that transcriptional regulation plays an important role in the regulation of mosquito *fer*, unlike their mammalian counterparts. Treatment with cyclohexamide also attenuated the expression of *fer* mRNA, though not to the same degree as treatment with actinomycin D. Taken together, these data suggest that the expression of mosquito *fer* is regulated both at the transcriptional and translational level.

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09-023

EXPRESSION AND FATE OF HEXAMERIC SERUM PROTEINS IN THE MOSQUITO (DIPTERA: CULICIDAE)

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All insects studied to date express one or more types of hexameric serum proteins or hexamerins during preadult development. We are examining the arylphorin-like proteins of mosquitoes to determine the roles of these aromatic-rich hexamerins in adult development, cuticle deposition and immune system responses. Higher Diptera express two major, immunologically distinct arylphorin-like proteins, LSP1 and LSP2, during the last larval instar. In a previous characterization of hexamerin holoproteins from *Aedes aegypti* and *Anopheles gambiae* we showed that, in contrast, fourth-instar (L4) mosquito larvae express heterohexamers composed of both LSP1- and LSP2-like subunits. Using polyclonal antisera purified against the two major *Aedes* arylphorin-like subunits, Hex1 and Hex2, we have determined their appearance in L4 hemolymph and their utilization during the pupal and adult stages. In *A. aegypti*, Hex1 and Hex2 disappear completely from adult hemolymph by day 3 post-eclosion. The effect of nematode parasitism and the blood meal on larval and adult hexamerin expression in both anopheline and culicine mosquitoes is being examined. We have previously isolated cDNA clones encoding the *Aedes* Hex1 and Hex2 subunits, and more recently a cDNA for an arylphorin-like protein of *A. gambiae*. The *Anopheles* clone encodes an 83.5 kDa polypeptide, most closely related to the higher dipteran calliphorin and *Drosophila* LSP1 proteins; we note the absence of an insertion characteristic of these higher dipteran hexamerins at the junction of domains 1 and 2. We will report the sequence analysis and comparison of hexamerin cDNAs for both culicine and anopheline species. We are currently isolating genomic clones for both *Aedes* and *Anopheles* hexamerin genes with a view to identifying their tissue-specific promoters and enhancers.

09-024

GS3, A GENE ISOLATED IN THE COCKROACH *BLATTA ORIENTALIS*, MAY REPRESENT A COUNTERPART OF THE VERTEBRATE NEURO-HEMATOPOIETIC SUPERFAMILY

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Despite the obvious morpho-physiological differences between insects and vertebrates, a number of regulatory and structural genes have been shown to play similar roles in both systems, providing the evidence that a common genetic program underlies the basic developmental decisions. Moreover, it has been suggested that a major factor in the evolution of eukaryotic gene families are gene duplications. As a consequence, paralogous genes could acquire new functions either by accumulating mutations and/or being recruited by different regulatory systems. A possible example of this tenet is the recently defined "neuro-hematopoietic factors" superfamily in vertebrates. A number of factors comprising neuro- (e.g. CNTF) and hematopoietins (e.g. IL6) have been ascribed to this superfamily, along with cytokines characterized by pleiotropy and functional redundancy (e.g. LIF/CDF).

By using a segment of the rat CNTF gene as a probe in Southern blotting experiments, we revealed the presence of positive signals in several insects. PCR amplifications using a number of mammalian-specific primers produced a segment from *B. orientalis* whose amino acid sequence shares similarities with the mammalian neuro-hematopoietic factors. Southern blot hybridization using this latter segment as a probe revealed that it represents a single-copy gene. Furthermore, Northern blotting analysis and RT/PCR experiments with cockroach-specific primers showed the existence of a transcript in all the body regions tested (head, thorax, abdomen). The full-length cDNA was obtained by using the RACE protocol. GS3 shares a degree of amino acid sequence similarity with the mammalian neuro-hematopoietic factors (10-14% amino acid identity and 36-38% conservative substitutions) comparable to that observed among different members of the vertebrate superfamily (e.g. 15% amino acid identity between human LIF/CDF and CNTF).

Further characterizations will require cloning of the GS3 gene in cockroach and other insects, and assessment of its pattern of developmental- or tissue-specific expression. However, it could be hypothesized the existence in insects of some trophic or differentiation factors related to the mammalian superfamily. If confirmed, new prospects to the interpretation of developmental mechanisms such as neurogenesis or hematopoiesis could be opened.

09-026

AN INTEGRATED GENOMIC MAP OF THE MALARIA MOSQUITO, *ANOPHELES GAMBIAE*, AND ITS USE IN IDENTIFYING *PLASMODIUM*-REFRACTORINESS GENES

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We have developed a dense genetic map of *An. gambiae* (average resolution 1.6 cM), partly in collaboration with the laboratories of F.H. Collins and C. Louis. The map consists largely of microsatellite (simple sequence repeat) markers, which are conveniently scored by PCR using a small fraction of DNA from each mosquito. Additionally, it incorporates RAPD, morphological and biochemical markers. A significant number of markers have been mapped by *in situ* hybridization to polytene chromosomes, thus correlating the map with cytogenetic locations. The map has been used in a collaboration with F.H. Collins, to identify and localize two genetic loci that are involved in refractoriness of *An. gambiae* to *Plasmodium*, based on encapsulation of oocytes. The map has also been used, in collaboration with K. Vernick, to identify and map preliminarily a third locus, which is involved in the refractoriness of *An. gambiae* to *P. gallinaceum*, based on lysis of ookinates in transit through the midgut epithelium. Prospects for molecular cloning of these genes and other phenotypically interesting loci will be discussed.

09-025

INSECT GENOMICS: PERSPECTIVES FROM *DROSOPHILA*

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Since the beginnings of this century *Drosophila melanogaster* has been the workhorse of genetics. The wealth of knowledge accumulated is unequalled by information on any other higher animal, and the development of molecular tools through the research on the fruitfly has promoted the knowledge on the development and genetics of other organisms from bacteria to man. It is now a well established fact that without the availability of genomic maps (initiated by Morgan on the genetic and Bridges on the cytogenetic level), this progress would have been unthinkable. The last few years has seen, through the advent of molecular techniques, an increased effort to establish for *Drosophila* also an integrated physical map that can be linked to the existing ones. The development of one such map by the European *Drosophila* Mapping Project (based on cosmid contig mapping and STS analysis) will be presented and the possible utilisation of the data for the study of other insects will be discussed.

09-027

A BACTERIAL ARTIFICIAL CHROMOSOME LIBRARY FOR THE MALARIA VECTOR *ANOPHELES GAMBIAE*

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A Bacterial Artificial Chromosome (BAC) genomic library for the malaria vector *Anopheles gambiae* has been constructed. This library contains 12,288 clones, representing approximately four genomic equivalents. Inserts in 100 randomly selected clones ranged from 70 kb to 145 kb, with an average insert size of 110 kb. Five clones with inserts larger than 110 kb were stable when grown for more than 100 generations in liquid media. No chimeric clones were detected among 40 BAC clones mapped by *in situ* hybridization to the *A. gambiae* polytene chromosomes. This library will be particularly useful for positional cloning applications.

09-028

SSCP ANALYSIS FOR RAPID LINKAGE MAPPING

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Analysis of genomes via Random Amplified Polymorphic DNA (RAPD-PCR) allows efficient construction of saturated linkage maps. When analyzed by agarose gel electrophoresis, most RAPD markers segregate as dominant alleles, reducing the amount of linkage information obtained. We have developed a mapping method based upon the use of Single Strand Conformation Polymorphism (SSCP) analysis of RAPD markers via polyacrylamide gel electrophoresis (RAPD-SSCP). We have used RAPD-SSCP to generate linkage maps in a haplodiploid parasitic wasp (*Bracon (Habrobracon) hebetor*) and two species of diploid mosquitos (*Aedes*). RAPD-SSCP analysis reveals segregation of codominant alleles at markers that appeared to segregate as dominant (band presence/band absence) markers or appeared invariant on agarose gels. Our SSCP protocol uses silver staining of large thin polyacrylamide gels and reveals more polymorphisms than agarose gel electrophoresis. The use of this method allows creation of saturated linkage maps in 6-10 weeks that are comparable to linkage maps created by alternative means (e.g. RFLP and visible mutations). Forty five percent of markers segregated as codominant loci in *B. hebetor*, while 11% segregated as codominant loci in *Aedes*. SSCP analysis of RAPD-PCR markers offers a rapid and inexpensive means of constructing intensive linkage maps of many species.

09-029

CONSTRUCTING A MOLECULAR LINKAGE MAP FOR THE DOMESTICATED SILKWORM, *BOMBYX MORI*M. R. Goldsmith, J. Shi, D. G. Heckel¹, D. R. Mills, N. Lawson, S. W. Marino, J. Leri, M. A. Choquette.

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We are constructing a linkage map for the silkworm based on restriction fragment length polymorphisms (RFLPs) using as probes anonymous cDNAs derived from an early follicular library, low copy number (10-15) repeats, and cloned sequences of known genes. A number of these markers serve as reference points to the conventional linkage maps. The initial RFLP map of 413 cM was constructed with a single F2 cross, and covers 15 linkage groups and 8 unlinked loci representing 23 of the 28 chromosomes. We are looking for additional linkage groups by means of backcrosses using heterozygous females, which have no crossing over, and homozygous males, followed by confirmation of linkage relationships and establishment of map position using additional F2 crosses. This scheme uncovered significant polymorphism in a genetically improved strain, C108, despite more than 20 generations of inbreeding in the laboratory.

Pilot studies are also underway to investigate the feasibility of mapping quantitative trait loci (QTLs) in this species, such as duration of the last larval instar, time to emergence, and pupal and cocoon weights. A known sex-linked gene, *Lm* (late maturity; 1-2.0), which has a major affect on these characters, is serving as an internal control.

09-030

LINKAGE MAPPING IN LEPIDOPTERA: TECHNICAL CHALLENGES, PRACTICAL APPLICATIONS, AND COMPARATIVE GENOMICS

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Construction of linkage maps in Lepidoptera is complicated by the large number of small chromosomes and the lack of general computational techniques to account for achiasmatic oogenesis in females. But progress has been greatly facilitated by development of molecular markers based on DNA polymorphisms, and the extension of the lod score method to deal with the unique genetic system of Lepidoptera. We describe the current status of the linkage map of the tobacco budworm *Heliothis virescens* (F), a polyphagous noctuid crop pest, and its application to localizing genes conferring resistance to chemical insecticides and endotoxins from *Bacillus thuringiensis* (Bt). Recently-developed computational methods and molecular techniques have also aided linkage mapping in *Bombyx mori*, *Yponomeuta padellus*, *Plutella xylostella*, and *Helicoverpa armigera*. The prospects for comparing the linkage maps of these Lepidoptera are discussed, and a system of universal anchor loci is proposed for this purpose. Comparative linkage mapping will not only shed new light on genomic evolution in the Lepidoptera, it will facilitate identification of homologous loci conferring resistance to Bt, an urgent concern in the pests *H. virescens*, *P. xylostella*, and *H. armigera*. It will also enhance the utility of the well-studied *B. mori* as a model for physiological and developmental processes in other Lepidoptera.

09-031

GENOME ANALYSIS AND MAPPING OF BEHAVIORAL TRAIT LOCI IN THE HONEY BEE

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Detailed linkage maps of the honey bee, *Apis mellifera* L., have been constructed by analyzing the segregation of RAPD markers (random amplified polymorphic DNA) in haploid males. Although the honey bee has a genome that is similar to *Drosophila* in the amount of repetitive and total DNA, the size of the linkage map indicates a very high rate of meiotic recombination. The crossover rate in the honey bee is higher than that reported for other higher eukaryotes and will enable map-based cloning of genes.

The maps have been used to identify loci with effects on behavioral traits. Two loci that influenced the resource choice of foragers (pollen versus nectar) were mapped. Loci that contributed to the propensity to sting in crosses with bees of African descent (*A. m. scutellata*) were also identified. The genetic analyses were performed on haploid male honey bees and the inheritance of RAPD marker alleles was then correlated with the behavior of the female progeny in colonies that these males fathered. Sequence-tagged sites (STS) have been developed that are linked to genes that affect pollen-foraging and stinging behaviors. These STS markers will be useful for mapping and studying gene effects in other populations of bees.

09-032

BIOSYNTHESIS, DISTRIBUTION, AND MODE OF ACTION OF PBAN IN *HELICOVERPA ZEA* (LEPIDOPTERA: NOCTUIDAE).

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Pheromone production in most moths is regulated by pheromone biosynthesis activating neuropeptides (PBAN). Biosynthesis and processing of the PBAN gene occurs in three sets of cells in the subesophageal ganglion (SEG). PBAN and 4 other neuropeptides from the same gene are expressed in the SEG and distributed to the corpora cardiaca in adult females. PBAN interacts directly with receptors on the pheromone gland. We will review the current concepts related to how PBAN stimulates the pheromone biosynthetic pathway at the level of the pheromone gland cell. Evidence will be presented for the role of calcium and cAMP in the signal transduction process. Current concepts on the signal transduction pathways involved in PBAN mode of action will be discussed.

09-034

NOVEL NEUROPEPTIDES FROM THE DESERT LOCUST, *SCHISTOCERCA GREGARIA*.

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By immunocytochemical staining we first demonstrated the presence of allatostatin-like material in the corpora allata of the locust. Next, immunopositive material was chromatographically purified from an extract of 7000 brains of *Schistocerca*. This yielded 9 peptides, which were named "schistostatins". They belong to the allatostatin A family, first isolated from the cockroach *Diploptera punctata*. One of these peptides, schistostatin-2¹¹⁻¹⁸, is the only purified peptide of this family that has no effect on juvenile hormone biosynthesis in the cockroach. The endogenous juvenile hormone production by isolated corpora allata of *Schistocerca* is very low. Perhaps, this is a reason why we were not able to demonstrate a clear inhibiting effect of the schistostatins in this assay system. *Locusta*-myoinhibiting peptide on the other hand was active at high concentrations. Schistostatins and *Locusta*-myoinhibiting peptide are potent inhibitors of spontaneous contractions of locust oviducts. Schistostatins are present in the hemolymph and their titer changes during certain developmental stages. Upon injection into adults, the schistostatins induce the appearance of an as yet only partially characterised 88 kDa protein in the hemolymph. The schistostatin preprohormone was cloned. It encodes ten putative peptides of which 8 were purified by HPLC. We also purified a peptide which seems to be a potent releasing factor of adipokinetic hormone from the corpus cardiacum of both *Locusta migratoria* and *Schistocerca gregaria*.

09-033

MOLECULAR BIOLOGICAL AND SIGNAL TRANSDUCING ASPECTS OF INSECT ADIPOKINETIC HORMONES

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Neurosecretory cells in the corpora cardiaca (CC) of the migratory locust, *Locusta migratoria*, synthesize three adipokinetic hormones (AKHs) I, II and III. In the adult locust, AKHs I and II are released into the hemolymph during flight and stimulate the release of carbohydrate (via activation of glycogen phosphorylase [GPh]) and lipid (probably via activation of triacylglycerol lipase) from the fat body. The released substrates serve for energy generation in the flight muscles. Data on release of AKH III during flight are lacking so far; however, injected AKH III reveals actions similar to those of AKHs I and II.

To date, information on the mRNA and/or gene structure of AKHs I and II is restricted to *Schistocerca* and *Manduca* species, whereas information on mRNA and/or gene structure of AKH III (which has been only identified in *Locusta* so far) is not available at all. For the migratory locust, we report the cDNA sequences encoding the three different AKH precursors. The AKH I and II precursors of *L. migratoria* are highly homologous to their *S. gregaria* and *S. nitans* counterparts, whereas the AKH III precursor of *L. migratoria* is more homologous to the *M. sexta* AKH precursor. *In situ* hybridization studies are presented, revealing the sites of expression of the three different AKH genes. In addition, we show that flight activity increases the three different AKH mRNA steady-state levels.

Since most neuropeptide receptors are members of the family of G protein-coupled receptors, we argued that AKH receptors may also be coupled to G proteins. To investigate this, the G_s activator cholera toxin (CTX) and the G_i inhibitor pertussis toxin (PTX) were applied to isolated fat bodies. Whereas CTX enhances both cAMP levels and GPh activity *in vitro*, PTX has no effect. Increases induced by CTX and one of the AKHs are not additive, suggesting them to share the same signalling pathway, namely via a G_s protein. Moreover, the G protein inhibitor GDPβS strongly attenuates GPh activation by the three AKHs, indicating that the receptor(s) for the AKH(s) are coupled to G_s.

In order to isolate and characterize the AKH receptor(s), we recently started with an expression cloning strategy using a colorimetric assay for measuring activation of G_s- and G_q- coupled signalling pathways.

09-035

SYNTHESIS AND EXPRESSION OF A GENE ENCODING A PUTATIVE INSECT NEUROPEPTIDE PRECURSOR IN TOBACCO

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The use of insect neuropeptides as an innovative approach for more effective strategy of integrated pest management is a very attractive perspective as neuropeptides are able to interfere with several insect physiological processes. In order to assess the feasibility of such approach a model peptide, proctolin, was selected for a preliminary set of experiments. Proctolin oral activity and gut absorption rate were evaluated in *Helicoverpa armigera* last instar larvae: the peptide proved to be orally active with an absorption rate of about 0.1-0.5%. A synthetic gene encoding for a putative proctolin precursor containing multiple copies of the neuropeptide in tandem array was assembled and subcloned in a plant expression vector later mobilized in *Agrobacterium tumefaciens* and used to transform tobacco plants. Phenotypically normal transformants with single or multiple insertions, were genetically stable over several generations and showed different level of expression of the synthetic gene.

09-036

EXPRESSION OF TRYPSIN MODULATING OOSTATIC FACTOR ON THE COAT PROTEIN OF TOBACCO MOSAIC VIRUS: A NEW BIORATIONAL INSECTICIDE AGAINST MOSQUITO LARVAE

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Trypsin modulating oostatic factor (TMOF), a decapeptide that modulates trypsin biosynthesis in the midgut of female mosquito was cloned into the carboxyl terminal sequence of the coat protein of Tobacco Mosaic Virus (TMV) with a trypsin cleavage site. Feeding TMOF to adult mosquito mixed with the blood meal stopped trypsin biosynthesis and egg development. Feeding mosquito larvae with TMOF or TMV-TMOF complex stopped larval growth and caused larval death. The mechanism and the mode of action of TMOF and its analogs on trypsin biosynthesis, cloning and expression and 3 dimensional molecular modeling of the TMV-TMOF complex will be discussed.

09-037

CLONING OF THE LOCUST (*SCHISTOCERCA GREGARIA*) ALLATOSTATIN PRECURSOR cDNA

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In insects, juvenile hormone biosynthesis (in the corpora allata) seems to be regulated by peptides originating from the nervous system. Members of the allatostatin A peptide family (the -FGLa AST-family) were first identified in cockroach species and have now been isolated from a number of distinct insect orders. Recently, we purified and sequenced nine AST-immunopositive peptides from the desert locust *Schistocerca gregaria* and called them schistostatins. One of these, schistostatin-2¹¹⁻¹⁸, is a truncated product of schistostatin-2.

Based on the peptide structures, we cloned the prepro-schistostatin cDNA. This sequence encodes a polypeptide which contains ten distinct AST variants. All purified schistostatins are found in this single precursor. Its organization displays some remarkable differences when compared to the ones known from cockroach species. Expression of the schistostatin gene in different locust tissues was monitored by northern analysis.

09-038

MULTIPLE SUBFAMILIES OF MARINER-LIKE TRANSPOSABLE ELEMENTS IN THE TEPHRITID FRUIT FLY *BACTROCERA TRYONI*

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DNA sequences related to the transposable element *mariner* have been found to be abundant in the genome of the Queensland fruit fly, *Bactrocera tryoni*, a tephritid species endemic to Australia where it is a serious pest of commercial fruit production. Multiple PCR fragments were cloned following their amplification from genomic DNA by use of degenerate oligonucleotide primers designed to amino acid sequences conserved between *mariner*-like transposases. DNA sequence variants from the cloned *B. tryoni* fragments can be grouped into at least two subfamilies of *mariner*-like transposase genes.

Representatives of each sequence class were used as probes in the screening of a *B. tryoni* genomic DNA library in lambda phage, resulting in a very large number of hybridizing clones. *B. tryoni* elements characterized from several independent positive clones were 90-95% similar to each other with partially conserved inverted terminal repeats (ITRs) of 30bp. These elements shared an overall DNA sequence identity of about 65% with previously characterized members of the 'mellifera' subfamily. All characterized *B. tryoni* elements appear non-functional, with transposase gene regions containing deletions and/or frameshift mutations.

To investigate whether the *B. tryoni* genome could currently support the transposition of *mariner*-like elements, plasmid-based transient assays were used. Microinjected constructs containing the related *Drosophila mauritiana mariner Mos1* ITRs underwent excision and transposition at a low frequency within *B. tryoni* embryos when an exogenous source of *Mos1* transposase was co-injected.

09-040

DISTRIBUTION OF *T1* AND *PEGASUS* TRANSPOSABLE ELEMENTS IN A NATURAL POPULATION OF *AN.GAMBIAE*.

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An.gambiae is becoming a target for genetic studies because of its importance as principal vector of human malaria in sub-Saharan Africa. Investigations of transposable elements are an important component of these studies because of their potential as vehicles for germline transformation and population genetic markers. Previously we have reported the chromosomal locations and stability of four transposable elements (*T1*, *Q*, *mariner* and *Pegasus*) in a laboratory strain of *An.gambiae*. There is no direct evidence that any of these elements are currently mobile, but *Pegasus* showed relatively high levels of polymorphism. To assess the degree of polymorphism in wild mosquito populations, we extended our study to a population of *An.gambiae* sampled from western Kenya. Using *T1* and *Pegasus* probes, we found that both transposable elements are polymorphic for all five chromosomal arms (except for *Pegasus* which has only one fixed site on X-arm), with average site occupancies of 0.44 and 0.26, respectively. In addition, the total number of copies differed between individuals, ranging from 29 to 43 for *Pegasus*, and 65 to 108 for *T1*. The reasons for this remarkable degree of polymorphism are being investigated.

09-039

MINOS TRANSPOSASE INDUCED INTERPLASMID TRANSPOSITION IN *D. Melanogaster* S2 CELLS

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Minos, a *Tc1*-like element from *Drosophila hydei*, encodes a transposase in two exons split by a 60 bp intron. This small intron is correctly spliced in *Drosophila melanogaster* carrying a single copy of a modified *Minos* element in which the transposase gene has been placed under heat-shock promoter control (Franz *et al.* 1994). The spliced message encodes for a protein that has been shown to have the properties of a transposase:

1) It can catalyze precise insertion of *Minos* transposons in the *D. melanogaster* (Loukeris *et al.* 1995a) and in the *Ceratitis capitata* genome (Loukeris *et al.* 1995b) in flies co-injected with helper and transposon plasmids at the preblastoderm stage.

2) It induces excision and transposition of these insertions in *D. melanogaster* transformants both in somatic and germ line cells (Loukeris *et al.* 1995a; Arca *et al.* submitted).

A system that would permit quick and efficient evaluation of new and modified tools for transformation, such as transposon and sources of transposase, would be of great help in designing such tools. Based on previous observation that *P* elements can excise, in the presence of transposase, not only from resident sites in the genome, but also from plasmids introduced into embryos or cell lines (Rio *et al.* 1986, O' Brochta and Handler, 1988; Rio *et al.* 1988), we have established a "transient excision" assay for *Minos* using *D. melanogaster* Schneider (S2) cells. We have also established a "transient interplasmid transposition assay" for *Minos* using the sucrose gene as a target (O' Brochta *et al.* 1994).

The transposition assay is based on the co-transfection of 3 plasmids: a "helper plasmid" coding for the transposase, a "donor" plasmid carrying a genetically marked, non-autonomous transposon, and a third plasmid carrying the sucrose gene. Transpositions of the marked transposon into the sucrose gene are selected for growth in the presence of sucrose and the appropriate antibiotics. Several events have been isolated and characterized at the molecular level.

The assay is used to study a) the properties of engineered transposases in vivo, b) the cis-acting DNA sequence requirements for *Minos* transposition, and c) transposition of *Minos* in different species.

09-041

ISOLATION AND ANALYSIS OF MARINER ELEMENTS IN THE TEPHRITID FLIES, *CERATITIS CAPITATA*, *C. ROSA* AND *TRIRHITHRUM COFFEA*

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Mariner transposable elements were isolated from three Tephritid species, *Ceratitis capitata*, *C. rosa* and *Trirhithrum coffea*. These species are sympatric in parts of their ranges, in particular, in Kenya where they are a pest of coffee.

The *mariner* elements were amplified by PCR using Inverted Terminal Repeat sequences (see accompanying posters of Gomulski *et al.*) and internal conserved regions as primers. Using the ITR primer, PCR products of around 1.3 Kb, were amplified in each species. These were cloned and sequenced. A high degree of similarity was found between the elements from each species. Southern blot analysis of genomic DNA indicates high copy numbers in these species.

We investigate the phylogenetic relationships of these elements with those from other species. The phylogeny of the elements is compared with the phylogeny of the species.

09-042

ANOPHELES GAMBIAE : EVIDENCE OF TYPE II TRANSPOSONS

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We are interested in characterising Type II transposable genetic elements (TGEs) in *An. gambiae*. We have employed the polymerase chain reaction (PCR) to amplify sequences homologous to *Tc1*-like TGEs based on degenerate primers. Using these primers with an *An. gambiae* DNA template a 384 base pair sequence has been generated and cloned (designated TRANG1). Cloned TRANG1 demonstrates significant sequence homology to the transposase gene of *Tc1*-like elements. The existence of sequences homologous to TRANG1 in closely related mosquito strains, variation in copy number and location have been suggested by the probing of genomic Southern blots. Screening of a representative *An. gambiae* genomic library with cloned TRANG1 fragment has identified 4 genomic clones. After Southern blotting, the fragments showing homology to TRANG1 have been subcloned into plasmid vectors and are being sequenced. In order to widen the search for divergent *Tc1*-like elements the entire genomic PCR product generated by the degenerate primers has been used to identify 15 additional genomic clones.

09-043

THE HERMES TRANSPOSABLE GENETIC ELEMENT AS A POTENTIAL MOSQUITO TRANSFORMATION VECTOR

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The lack of a reliable method to transfer genes into insects of medical importance still represents a major stumbling block in the development of transgenic technology. Until such procedures are routine, progress in the molecular genetic analysis of these insect systems and the exploitation of transgenic technology will be slow. In order to assess their potential as the core of a mosquito transformation system we are currently testing the integration efficiency of the housefly (*Musca domestica*) *hermes* transposable genetic element in the genome of *Aedes aegypti*. Two approaches are being undertaken. Firstly, to detect *hermes* integration, a two element vector/helper system, comprising of the *hermes* open reading frame controlled by the *Drosophila hsp70* heat-shock promoter and a marked *hermes* element, containing the *opd* gene controlled by the same promoter, are co-microinjected into early *Ae. aegypti* embryos. Potential G1 transformed offspring are selected using the nerve agent paraoxon. Southern blotting and sequencing are used to determine the nature of any integration events. Secondly, to detect transposition of *hermes* in the mosquito cytoplasm, a similar vector/helper system, containing the bacterial marker kanamycin, are coinjected with the target sucrose gene. In order to detect possible transposition events, rescued plasmid from 24 hour old heat-shocked embryos are transformed into *E. coli*, kanamycin resistant sucrose sensitive colonies are selected. Southern blotting and sequencing of rescued plasmid DNA are being used to determine transposition events. The results from these experiments will be described and discussed in relation to whether *hermes* has potential as the core of a mosquito transformation vector system.

09-044

ZEBEDEE1: A NOVEL COPIA-TY1 TRANSPOSABLE ELEMENT IN THE GENOME OF THE MEDICALLY IMPORTANT MOSQUITO, Aedes Aegypti

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We have utilised PCR to directly identify a novel *cop*ia-Ty1 retrotransposon element, *zebedee1*, in the genome of the mosquito *Aedes aegypti*. *Zebedee1* is 3505 bp long and may be flanked by 21 bp direct terminal repeat sequences. A single open reading frame (ORF) of 972 amino acids has the coding potential for a polyprotein with homology corresponding to the conserved amino acid motifs of Long Terminal Repeat (LTR)-retrotransposon protease, integrase and reverse transcriptase. There appear to be less than 10 copies of *zebedee1* in the genomes of different strains of *Ae. aegypti*, and transcripts of the element have been detected in cultured mosquito cells by RT-PCR. Despite the lack of LTRs or a *gag* homologue, phylogenetic analyses place *zebedee1* within the *cop*ia-Ty1 group of LTR retrotransposons, showing significant homology to *cop*ia from *D. melanogaster*. Interestingly, in one case we have identified a new mosquito LINE (Long Interspersed Nucleotide Element), *JAM1*, integrated in close proximity to *zebedee1* (see Hughes *et al.*, accompanying poster). This element is downstream of *zebedee1* and has an opposite polarity.

09-045

JAM1: A NOVEL LINE TRANSPOSABLE ELEMENT IN THE GENOME OF THE MEDICALLY IMPORTANT MOSQUITO, Aedes Aegypti

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We have utilised PCR to directly identify a novel *cop*ia-Ty1 retrotransposon element, *zebedee1*, in the genome of the mosquito *Aedes aegypti* (see accompanying poster by Warren *et al.*). Interestingly, during this search we have identified a new mosquito LINE (Long Interspersed Nucleotide Element), *JAM1*, integrated in close proximity to *zebedee1*. This element is downstream of *zebedee1* and has an opposite polarity. *JAM1* is flanked by imperfect 20 bp repeats and is 3364 bp long with two ORFs; ORF1 codes for a polypeptide of 176 amino acids long and contains a copy of the nucleic acid binding motif; ORF2 codes for a polypeptide 835 amino acids long and has homology to LINE-like reverse transcriptases. Many copies (~1,000) of *JAM1* are present in different strains of *Ae. aegypti*, and an abundant transcript of ~7 kb homologous to the element has been detected in cultured mosquito cells. Neither long, 3' poly A stretches or host direct flanking sequences at the site of integration in *zebedee1* are observed.

09-046

TRANSPOSABLE ELEMENTS COULD BECOME TARGETS ONE ANOTHER FOR INSERTION

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Six to nine fragments from 0.6 to 4.2kb, were amplified in genomic DNA of *Bombyx mori* by PCR using inverted repeat of transposon, mariner, from *Hyalophora cecropia* as a primer. Almost all bands were observed in eight tested strains of *B. mori*. The longest fragment of 4.2kb was cloned from PCR products of the N4 strain as a plasmid, pBmTNER1, and sequenced. This clone contained mariner (1.2kb), a known *B. mori* retroposon, Bm2 (0.2kb), and a retrotransposon, BMC1 (2.8kb). The sequence data revealed that Bm2 had been integrated into mariner, and then BMC1 had been integrated into Bm2 with a target sequence duplication at each flanking region. The isolated mariner whose copy number is less than 50, was a defective type containing no ORFs except for the inserted region of Bm2 and BMC1. As the consensus sequences conserved in wide spread mariner families were unable to amplify any fragments in *B. mori* by PCR, there may not be any active mariners. These data suggest that transposable elements could be available to one another as targets for insertion in *B. mori*.

09-048

INCREASED SURVIVORSHIP AND EXPRESSION OF FOREIGN RNA AMONG MICROINJECTED PINK BOLLWORM EMBRYOS (LEPIDOPTERA: GELECHIIDAE).

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mRNA transcribed *in vitro* was introduced into *Pectinophora gossypiella* embryos by microinjection. We have optimized methods to increase survivorship and demonstrated translation of injected RNA into functional protein. Here, we report a five-fold increase in survivorship with the electromechanical Eppendorf 5171 system over a manually operated Narashige unit and the use of β -galactosidase as a positive control for translation.

09-047

THE GENOMES OF MOST INSECTS AND OTHER ANIMALS HAVE MULTIPLE MEMBERS OF THE *Tc1* FAMILY OF TRANSPOSABLE ELEMENTS

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A PCR assay was employed to detect sequences homologous to the transposase gene of the *Tc1* family of transposable elements in a wide variety of insects and other animals. Amplification products of the appropriate size were obtained from most insects (92 of 108 examined; 85%), most other invertebrates (33 of 43; 77%), and many vertebrates (18 of 36; 50%). Sequencing of a sample of cloned PCR products from eight insects, one hydra, and two frogs revealed that each had multiple distinct members of the family in their genomes. In the most extreme case, the horn fly *Haematobia irritans* yielded evidence of seventeen distinct types of *Tc1* family elements. Most of the sequences obtained indicate that the elements are within the range of variation already known from fungi, nematodes, flies, fish, and frogs, however some had novel length variants or extremely divergent sequences, indicating that they represent new subfamilies of these transposons. These results indicate that this family of transposons is extremely common in animal genomes, with multiple representatives in most genomes.

09-049

VITELLINE MEMBRANE GENES IN *Aedes Aegypti* MOSQUITOES

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A genomic clone corresponding to a novel *Aedes aegypti* vitelline membrane gene (15a-3) has been isolated. The predicted peptide sequence of 15a-3 is similar to those of the *A. aegypti* vitelline membrane genes 15a-1 and 15a-2. The deduced amino acid sequences of 15a-1, 15a-2 and 15a-3 contain a region of 45 residues which is highly conserved between the three genes. The conserved region overlaps with a region that is conserved between four *Drosophila melanogaster* vitelline membrane genes. DNA regions of approximately 1.8 kb, 2.1 kb and 2.0 kb were sequenced upstream of the 15a-1, 15a-2 and 15a-3 coding regions respectively. Sequences with similarity to a 20-hydroxyecdysone response element consensus sequence were found upstream of the 15a-1, 15a-2 and 15a-3 coding regions. The transcriptional regulation 15a-1, 15a-2 and 15a-3 was examined. The respective timing and sites of expression of the three transcripts were determined by Northern blot analysis and whole-mount *in situ* hybridization using mutually exclusive probes. The temporal pattern of 15a-1, 15a-2 and 15a-3 expression was similar. The spatial pattern of expression differed between the three genes. Only 15a-2 was expressed at the anterior region in addition to the remainder of the follicle. 15a-1 and 15a-3 were only expressed in the mid and posterior regions of the follicle. The effect of 10^{-5} M 20-hydroxyecdysone on vitelline membrane gene expression was examined *in vitro*. Expression of 15a-1 was higher in ovaries that were dissected at intervals of 0, 2, 10 and 24 h after a blood meal and were cultured in medium containing 20-hydroxyecdysone, as compared to control incubations. In ovaries that were dissected at 36 h after a blood meal, incubation in 20-hydroxyecdysone had no effect on 15a-1 expression, as compared to control incubations.

09-050

ORGANIZATION AND EVOLUTION OF AN AUTOSOMAL CHORION LOCUS IN THE MEDFLY *CERATITIS CAPITATA*

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Four autosomal chorion genes, Ccs16, Ccs19, Ccs15 and Ccs18, and one non-chorion gene (NC-ORF), have been mapped on a chromosomal region of 20 kb, in *Ceratitis Capitata*. The chorion genes are tandemly arranged and individually regulated as shown by the temporally distinct expression of each one. The fifth gene is organized in the opposite orientation close to the 3' end of Ccs16. The two last exons of this gene have been sequenced and showed clear homology to myosin-like genes. Structural and regulatory evolution of the autosomal chorion loci in four distantly related *Drosophila* species and *Ceratitis capitata*, showed that this cluster is remarkably conserved in overall organization: all five species have preserved the same four chorion genes in the same order (s18, s15, s19 and s16) and with variation in spacing: in *Drosophila* species, the distance from the s18 transcriptional start site to the end of the s16 genes ranges from 5.5 to 7.0 kb while in *C. Capitata* it has expanded to 13.5 kb. The direction of transcription is also invariant both, within and across species: all genes are transcribed from the same strand and are identically oriented relative to NC-ORF which belongs to the same linkage group with chorion genes, in all studied species. Chorion genes are also highly conserved in developmental properties: in all five species they amplify extensively in the follicular epithelial cells, and produce exclusively follicular transcripts with gene specific temporal regulation, very similar in *Drosophila* species but slightly altered in *C. Capitata*. In contrast to this organizational and regulatory conservation, chorion gene DNA showed extensive sequence diversification, although not homogenous in distribution. The coding regions of the genes show a high degree of sequence divergence especially in *C. Capitata*, although in the proximal 5' flanking gene regions of homologous elements may have a very important role in regulation of transcription and amplification.

09-052

CLONING AND EXPRESSION OF AN INSECT GLUTAMATE TRANSPORTER GENE

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In recent years, great progress has been made in cloning and characterizing a number of molecular transporters responsible for the uptake of excitatory amino acids in mammalian systems. Strong homologies observed in the amino acid sequences of these proteins have suggested the occurrence of several large transporter families which are widely conserved. Making use of the mammalian sequences, we designed oligonucleotide primers to amplify homologous DNA fragments from insect cDNA by PCR. By this method, a DNA fragment was isolated from a cDNA library constructed from heads of the caterpillar *Trichoplusia ni*. The fragment produced was then used as a hybridization probe to isolate larger cDNAs.

The complete sequence of a 1.7 kilobase cDNA clone has been obtained, and found to contain an open reading frame encoding a protein with high homology to one transporter family described above. The deduced protein sequence is 44% and 45% identical to known rabbit and human glutamate transporters, respectively.

Expression of the isolated cDNA in a baculovirus expression system has been used to determine the characteristics of this insect transporter. Infected insect cells were found to exhibit greatly increased transport of various structural analogues of glutamate as compared to uninfected cells. This activity was completely Na⁺-dependent, suggesting the likelihood that the cloned transporter represents an insect homologue of this recently recognized family of mammalian glutamate transporters.

09-051

HORMONAL REGULATION OF VITELLOGENIN GENE EXPRESSION AND ITS STRUCTURE IN HOUSEFLY, *MUSCA DOMESTICA*N. Agui, T. Shimada¹, S. Izumi² and S. Tomino²Department of Medical Entomology, The National Institute of Health Shinagawa-ku, Tokyo, Japan -¹ Department of Agricultural Environmental Biology, The University of Tokyo, Bunkyo-ku Tokyo, Japan -² Department of Biology, Metropolitan University, Hachioji-shi Tokyo, Japan

Ecdysteroids induced vitellogenin synthesis and its gene activation in the ovaries of females and in fatbodies of both sexes in the housefly. Juvenile hormone (JH) acts only on female fly for induction of vitellogenin gene transcription (Agui *et al.*, 1985, 1991). Furthermore, our results showed that treatment of the female flies with JHA *in vivo* and *in vitro* increased ecdysteroid levels in hemolymph and ovary. This increase may contribute to the induction of vitellogenin gene expression. Thus, the role of JH in the vitellogenin gene expression is indirect and is mediated through stimulation of ecdysone synthesis in ovary. The ecdysteroid-receptor concentration in females was higher than that of males as measured by receptor-binding assays.

The female specific expression of the vitellogenin gene may depend upon the significant differences in ecdysteroid titer and ecdysteroid receptor levels between female and male flies. Furthermore, the presence of the reproductive organ, ovary itself, greatly contributes to the sex-specific expression of the vitellogenin gene by producing ecdysone.

We represent here that the sequences of the housefly vitellogenin gene (Mvg), which was isolated from a housefly genomic DNA library using the Mvg cDNA probe. The coding nucleotide sequence of Mvg showed high similarity with those in *Ceratitis capitata*, *Calliphora erythrocephala* and *Drosophila melanogaster*. The intron of Mvg has no homology, although its size and location is similar to that of *Drosophila*. The upstream sequences of the 5' end also showed no similarities with other YP genes within 200 base pairs upstream.

09-053

JUVENILE HORMONE INDUCES TRANSCRIPTION OF A TRYPSIN GENE IN *Aedes aegypti* MIDGUT

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Two types of trypsin are involved in blood meal digestion in the yellow fever mosquito, *Aedes aegypti*. Early trypsin is synthesized in small amounts within the first 4-6 hours following a blood meal. It is responsible for the first phase of tryptic digestion, which involves tasting of the meal and transcriptional activation of the more abundant late trypsin form. Early trypsin is an adult female-specific protein; transcription of the early trypsin gene begins shortly after emergence, and the mRNA level dramatically increases during the first day following emergence. In this poster we show that early trypsin gene expression is regulated by JH. Abdominal ligation within one hour after emergence silenced early trypsin gene expression. One hundred pg of topically applied methoprene (JH analog) restored early trypsin gene expression in ligated abdomens to values of non-ligated controls. The induction was dose dependent and "head-independent." In addition, methoprene was able to induce expression of the early trypsin gene in pupae.

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09-054

GENE SEQUENCE AND ORGANISATION FOR A SEX LINKED ACETYLCHOLINESTERASE GENE IN INSECTICIDE RESISTANT *CULEX PIPPIENS*

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Acetylcholinesterase (AChE) hydrolyses the neurotransmitter acetylcholine. The study of AChE in pest insects is important since it is the target for carbamate and organophosphate insecticides. Resistance has developed to these insecticides in several pest species involving an insensitive AChE.

A PCR generated homologous probe for part of a *Culex pipiens* AChE gene was used to screen a genomic DNA library prepared from a strain homozygous for insensitive AChE. Eight clones were isolated. Restriction enzyme mapping showed that these corresponded to three distinct maps containing a conserved region within which the probe hybridised. Two overlapping clones have been shown to match a sex linked gene by RFLP analysis (Bourguet unpub.). To date ~15 kb of these clones has been sequenced. The complex AChE gene organisation found in other Dipterans was present. Intron sizes were large: more comparable with the *D. melanogaster* AChE gene than other mosquito species. However the additional intron found in *A. stephensi*, but not other Dipteran AChE genes was present. The predicted amino acid sequence shows considerable homology to other mosquito AChE genes. No substitutions likely to confer insecticide insensitivity were discovered which is consistent with evidence that the factor determining insensitivity is autosomal (Bourguet unpub.).

09-056

SEQUENCE OF A CDNA CLONE ENCODING THE MOSQUITO L18 RIBOSOMAL PROTEIN

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An *Aedes aegypti* cDNA clone encoding the ribosomal protein-encoding gene, L18, has been isolated and the sequence determined. The cDNA clone was found to contain 672 bp, including a coding region of 560 nucleotides (nt) and a 3' extension of 112 nt. Comparisons between the coding regions of this clone to other known L18 aa sequences indicate good homology and their relationship is represented in the form of a phylogenetic tree. Evidence is presented to show that the *Ae. aegypti* sequence is a single-copy gene and that a comparable and closely related sequence exists in the mosquito, *Anopheles gambiae*. This is the first report of an insect L18 ribosomal protein-encoding gene.

09-055

THE HEAT SHOCK 70 GENES OF THE MEDFLY *Ceratitis capitata*: GENE ORGANIZATION AND STRUCTURE

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The heat shock genes have been studied extensively in several organisms. The promoters of these genes are the best characterized highly induced DNA regulatory elements and they have been demonstrated to be important components of gene expression vectors. The cloning and characterization of the heat shock 70 (Hsp70) genes of the medfly *C. capitata* are presented in this work. Our goal is the development of medfly heat shock expression vectors which are anticipated to facilitate gene manipulation in this widespread and destructive insect pest.

Six genomic clones have been isolated by screening a medfly genomic library with an HSP70 genomic clone fragment of *D. melanogaster*. Restriction analysis indicated that the six clones are organized in two regions, approximately 30 kb each, and are mapped cytogenetically in one of the major heat shock puffs (3L:24C) of the salivary gland polytene chromosomes. Further analysis by a combination of restriction mapping and blot hybridization indicated the presence of six putative HSP70 genes in these two regions. One of these genes was further characterized by sequencing. Our data demonstrate that this gene is a heat shock inducible HSP70 gene: The coding region shows about 70% homology, at the nucleotide level, to the HSP70 genes of *D. melanogaster* and the 5' untranslated leader sequence (200 bp) is not interrupted by introns and is very rich (~48%) in adenine residues. Furthermore, the promoter of this gene contains two characteristic heat shock elements, 17 and 34 bp, upstream from the TATA box.

09-057

ANOPHELES GAMBIAE MIDGUT SPECIFIC GENES INDUCED BY A BLOOD MEAL: A DIFFERENTIAL DISPLAY ANALYSIS

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We are interested in identifying *Anopheles gambiae* genes involved in the penetration of *Plasmodium falciparum* ookinetes through the mosquito midgut. Characterization of such genes could permit the identification of targets or new means for blocking the development of the parasite in this host.

Using the technique developed by Liang and Pardee (1992), we initiated an extensive display of the genes specifically expressed in the midgut of *Anopheles gambiae* and induced or up-regulated after a blood meal. cDNAs corresponding to mRNAs expressed between 18 to 24 hours after the blood meal are selected and further characterized. The ones having the expected expression pattern will then be used to isolate full-length c-DNA's. We have currently selected 100 of such cDNA fragments. Verification of their expression pattern and determination of their partial sequence is under-way.

Liang, P., & Pardee, A. B. (1992). *Science*, 257 967-971.

09-058

DAY-LENGTH DEPENDENT GENE EXPRESSION OF JUVENILE HORMONE ESTERASE IN THE COLORADO POTATO BEETLE

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In the Colorado potato beetle, juvenile hormone (JH) plays a key role in day-length dependent physiological processes and it is the JH titer which determines whether reproduction or diapause will occur. One of the main factors, besides JH synthesis, which influences the JH titer is enzymatic degradation of JH by a specific esterase. Beetles reared under short-day conditions showed high JH esterase activity after the initial drop in JH titer. If the JH titer decreases to almost zero, the beetles will enter diapause. The JH esterase was isolated from the hemolymph and appeared to be a dimer consisting of 57 kDa subunits. The cDNA encoding JH esterase was cloned and used as probe to follow the expression of the gene in the fatbody. The level of mRNA was higher in short-day beetles than in long-day beetles, which corresponds with the enzyme activity.

09-059

CLONING AND EXPRESSION OF HEAT SHOCK PROTEIN GENE AT THE ONSET OF POST-DIAPAUSE DEVELOPMENT IN *BOMBYX MORI* (LEPIDOPTERA: BOMBICIDAE)E. Kosegawa, W. Hara¹, T. Kikawada, K. Shimizu and T. OkajimaDepartment of Genetics and Breeding, National Institute of Sericultural and Entomological Science, Yamanashi, Japan - ¹ Department of Research Planning and Coordination, National Institute of Sericultural and Entomological Science, Ibaraki, Japan

The silkworm, *Bombyx mori*, enter diapause at the early gastrula stage. The embryonic diapause can be artificially terminated by hot HCl treatment after the chilling for one month. Some proteins were found to be induced at the onset of post-diapause development after the hot HCl treatment (Coulon-Bublex and Dorel, 1991; Kosegawa et al., 1991). These include proteins which resemble typical heat shock protein HSP70 in terms of isoelectric point and molecular weight. Cloning of HSP70 cDNA and Northern analysis detected the HSP70 gene transcription in the artificially activated eggs. On the basis of the results obtained, the role of HSP70 on diapause termination will be discussed.

09-060

EXPRESSION PATTERNS AND CHROMOSOMAL LOCALISATION OF THE LATE TRYPSIN GENE IN *AEDES AEGYPTI*.F.O. Oduol¹, J.M. Crampton¹, D.W. Severson², P. Eggleston³.

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Currently, there is much interest in the development of transgenic technology to provide novel strategies for vector control through the manipulation of mosquito genomes. Ultimately, the successful exploitation of this approach may require some degree of control over transgene expression. It may, for example, be useful to have promoters which limit expression to certain tissues (such as the mid-gut) or to certain times (perhaps post blood meal). We are investigating the late trypsin gene from the mosquito *Aedes aegypti*, which is a serine protease expressed at high levels after a bloodmeal. Northern blot analysis of temporal expression patterns in the mid-gut reveals that late trypsin mRNA can be detected 8 hours after the bloodmeal, with maximal expression at 24 hours post-bloodmeal. Spatial expression patterns have also been investigated by hybridising histological sections of the mid-gut with late trypsin probes, at various time points after the bloodmeal. The late trypsin gene (*TyAD1*) has been genetically mapped using RFLP multipoint linkage analysis among F2 intercross individuals from a pairwise mating between a Liverpool strain female and a RED strain male. This analysis located *TyAD1* to chromosome 2 with the following linkage associations; *spot-abdomen* [17.3cM] *LF98* [3.7cM] *TyAD1*.

09-061

ORGANIZATION OF GENES CODING FOR MALE SPECIFIC SERUM PROTEINS IN THE MEDFLY *CERATITS CAPITATA*Christophidis G.¹, Thymianou² S., Mauroidis M.², Mintzas A.², and K. Komitopoulou¹¹ Department of Biology, Section of Biochemistry, Molecular and Cellular Biology and Genetics, Athens Greece² Department of Biology, Section of Genetics, Cell Biology and Development, Patras Greece

Nine genomic clones containing genes coding for male specific serum proteins have been isolated by screening a genomic library with an MSSP cDNA clone. Restriction mapping of them has revealed the existence of four MSSP gene copies, which are organized in two pairs, named a (a1 and a2) and b (b1 and b2). Each pair is contained within 8.5 kb and the two copies are tandemly arranged. Moreover an insertion of a mariner-like element has been found 320 bp upstream of the transcription site of a1 and a2 genes.

Subcloning and sequencing of appropriate restriction fragments containing each MSSP copy and its flanking sequences, showed that they have exactly the same organization: each one consists of two exons, one small and one large, separated by an intron 400 long. They have also small untranslated regions, 23 bp at the 5' end and 79 bp at the 3' end.

Comparison of DNA sequences containing the four MSSP genes showed a great similarity not only in coding regions but also in introns and the DNA surrounding the genes. The a2 MSSP gene is almost identical to the cDNA clone which has been used as a probe for the isolation of the genomic clones, with only one nucleotide substitution. Comparison of a1 and a2 copies at the DNA level showed 15 nucleotide substitutions in the coding region but 7 amino acid changes at the predicted polypeptide sequence. Comparison of b2 and a2 coding regions showed 38 nucleotide substitutions and an insertion of 3 nucleotides. These preliminary results showed that the MSSP genes belong to a small multigene family. The remarkable similarity at the DNA and protein level suggests that they have resulted from recent duplications.

09-062

THE EVOLUTION OF AN INTRON IN THE ACETYLCHOLINESTERASE GENES OF DIPTERA

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Acetylcholinesterase (AChE) hydrolyses the neurotransmitter acetylcholine. It is the target for carbamate and organophosphate insecticides. Resistance to these insecticides conferred by an insensitive form of AChE has developed in several pest insect species. This has stimulated studies on the molecular genetics of insect AChE genes. The DNA sequence and genomic organisation of several Dipteran AChE genes have been determined. In each case the gene has been complex with eight or nine introns. Despite up to ten fold variation in intron size between species, the exon sizes and exon intron boundaries are conserved. However in two species, *Anopheles stephensi* and *Culex pipiens*, an additional intron splits the exon corresponding to exon 4 in *Drosophila melanogaster*. In addition to *D. melanogaster*, the intron is absent from the AChE genes in *Ceratitus capitata* and *Anopheles albimanus*. It is this last finding which makes the results interesting, since *A. stephensi* is more closely related to *A. albimanus* than *C. pipiens*, it appears that the intron has either been lost from the gene in the other species, or that there have been two separate insertion events into an identical position within the gene. An alternative, but perhaps more intriguing explanation may be the possibility of two genes in some species.

These observations and a discussion of the various explanations will be presented. Further data now being accumulated on the presence or absence of this intron in AChE genes of other Dipteran insects for which sequence and/or clones are available will also be presented.

09-064

NEW ANEUPLOID STRAIN DERIVED FROM ANTENNAPEDIA MUTANT LINE, *Nc* OF *BOMBYX MORI* (LEPIDOPTERA : BOMBYCIDAE)

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We got a new abnormal morphological strain (*Nct*) derived from the *Nc* · *U Antennapedia* mutant line maintained in Laboratory of Genetic Resources (NISES) in the process of the detouching between 6th and 14th chromosome. This *Nct* larva forms the incomplete eye spot and crescent like *Nc* heterozygous larva. But *Nct* does not have embryonic lethality with the incomplete formation of the thoracic legs although *Nc* homozygous embryo does not hatch because of the incomplete formation of their thoracic part. Southern hybridization analysis of the genomic DNA extracted from the PSG of the individual larva with *Bombyx-Antennapedia* (*BmAntp*) cDNA and genomic probes showed that *Nct* larva has three doses regarding Antennapedia locus, namely two normal and one *Nc* locus which deletes the homeobox region. The mixed chromosomes constitution of *n*=28 and 29 was also observed in *Nct* testis cell. These results indicate that *Nct* is (partially?) trisomic line at least regarding *BmAntp* locus and suggest that the product of *Nc* locus may prevent the correct formation of the thoracic part even on the condition with the normal doses of the *Antennapedia* homeobox region.

09-063

SEQUENCE AND STRUCTURE OF THE DIPHENOL OXIDASE GENE IN *ANOPHELES STEPHENSI* AND ITS RELATIONSHIP TO AN UNSTABLE MELANOTIC MUTANT *BLACK LARVAE*

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The diphenol oxidase gene (*Dox 2A*) in *Drosophila melanogaster* is found within a densely packed region of at least 34 genes covering approximately 100 kb. Many melanotic mutants have been found within or close to these clusters. A study on *Anopheles gambiae* (P. Romans per comm.) suggests synteny conservation of the region.

A 4.3 kb *A. gambiae* genomic DNA clone containing a gene with homology to *Dox 2A* was used to screen a *A. stephensi* genomic DNA library. This identified a ~16 kb fragment containing a region hybridised by the whole probe. DNA sequence from part of the conserved region showed homology to *Dox 2A*: the complete gene (1.5 kb) gave a predicted amino acid sequence with 67% identity. The single intron present in *Dox 2A* was absent.

Melanotic mutants resulting in black larval body colour have been found several times in *An. stephensi*, but are often homozygous lethal. One viable mutant (*BI*), co-dominant with wild type, has been used as a linkage marker and to develop a genetic sexing mechanism. These studies determined its approximate position towards the centromere on chromosome arm 3R. This mutant showed occasional instability, suggesting an insertional mutation involving a transposable element where most the population showed mosaic black phenotypes and a few revertants to wild type. Re-selection for the full black phenotype took several generations, despite the co-dominance. The possibility that this mutant gene was in, or close to, the *Dox/Ddc* gene clusters was examined by *in situ* hybridisation of the *Dox* clone to polytene chromosomes. The result was consistent with this hypothesis and more detailed mapping studies are now in progress.

09-065

NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCE OF A cDNA CLONE ENCODING STORAGE PROTEIN-1 OF *HYPHANTRIA CUNEA* DRURY.

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N-terminal and peptide sequence were determined from purified storage protein-1 of *Hyphantria cunea* Drury. In order to clone the storage protein, the DNA probe was generated by PCR using two degenerate oligonucleotide primers encoding N-terminal region and region for residue 84-91. Using probe we isolated the cDNA clone of storage protein-1 from cDNA library. The deduced amino acid sequence was highly homologous to methionine rich storage protein of *Manduca sexta*, sex specific storage protein of *Bombyx mori*, and basic juvenile hormone suppressible protein of *Trichoplusia ni*.

09-066

Expression and the biochemical characterization of an *Anopheles dirus* gene encoding an insect class I glutathione S-transferase.

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We have used a 5' RACE (rapid amplification of cDNA ends) reaction with the primer designed from a region of high N-terminal sequence homology in *An. gambiae* GST-I enzymes to amplify a full-length glutathione S-transferase cDNA from *An. dirus*, a malaria vector in Thailand. This GST has high N-terminal homology with GST-I from *Drosophila melanogaster* and *Musca domestica*. The cDNA was cloned into PET vector for expression in E.coli and the resultant protein biochemically characterized. Characterization included steady state kinetics, substrate specificity and xenobiotic inhibition. The capability of the expressed enzyme to catalyze DDT-dehydrochlorination is compared with earlier studies on the partially purified isoenzymes from whole insect homogenates.

09-067

MOLECULAR EVOLUTION OF THE α -ESTERASE GENE CLUSTER THROUGH THE *DROSOPHILA* RADIATION

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We present a molecular analysis of the α -esterase cluster of *Drosophila melanogaster*. The cluster spans 60kb and contains 11 esterase genes, each of at least 1.6kb. Reconstruction of the gene phylogeny using the amino acid sequence of the esterases reveals three simple gene duplication events. Two of these resulted in the duplicate genes inverted with respect to each other, while the other resulted in a 'head-to-tail' arrangement. The most recent of the three duplications is particularly unusual because it involves non adjacent genes; one of which is now a pseudogene that is located in the intron of another esterase gene. Other features of the α -esterase cluster's evolution include: the diverse pattern of intron presence/absence that requires multiple independent intron gain/loss events, the apparent lack of recent gene conversion events, and the saturation of silent sites indicative of relatively old gene duplication events.

In order to further investigate the events and processes that have shaped the cluster, we have attempted to superimpose the gene phylogeny onto the well established phylogeny of *Drosophila* species. The α -esterase cluster of *D. buzzatii* contains at least nine of the esterase genes represented in *D. melanogaster*, demonstrating that these genes are at least as old as the divergence of the *Drosophila* and *Sophophora* subgenera (an estimated 30-60 million years). The pseudogene however is not present in the *D. buzzatii* cluster so to trace its evolution we have focused upon other species within the *Sophophora* subgenus. We are particularly interested in the timing of its inactivation because both relative rate tests and the pattern of amino acid replacements suggest that it may have been functional for some time after the gene duplication event.

Other genes in the α -esterase cluster can be traced back to at least the divergence of drosophilids and calliphorids (80-100 mya). In *Lucilia cuprina* (sheep blowfly) and probably *Musca domestica* (housefly), orthologs to the *D. melanogaster* α -esterases have been implicated in metabolic resistance to OP insecticides. There is also some evidence to suggest that the amplified esterase genes in OP-resistant *Culex* mosquitoes may be distant orthologs of the α -esterases of *D. melanogaster*.

09-068

SEQUENCE ANALYSIS OF THE RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER 2 IN PALEARCTIC SPECIES OF THE *ANOPHELES MACULIPENNIS* COMPLEX (DIPTERA: CULICIDAE).

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Mosquito rDNA shows typical eukaryotic structure and organisation in which multiple copies of the ribosomal genes are tandemly arranged. In general, comparison of nucleotide sequence of various portions of the ribosomal RNA has proved to be a useful molecular tool to studying evolutionary affiliations among taxa. Certain areas of rDNA evolve rapidly, while other areas tend to be highly conserved even in distantly related organisms, particularly the coding regions. Non-genic, internal transcribed spacers (ITSs) have been useful in elucidating phylogenetic relationships between species of mosquitoes, including sibling ones.

The present study was designed to study the ITS-2 (internal transcribed spacer-2) sequence variation in Palearctic species of the *An. maculipennis* complex, i. e. *Anopheles atroparvus*, *An. maculipennis*, *An. labranchiae*, *An. messeae* and *An. sacharovi*, which includes both potential malaria vectors and non-vectors sibling species, in order to elucidate their phylogenetic relationships (speciation within the group is probably of recent origin) and to compare the phylogeny so inferred with those resulting from the morphological characters, polytene chromosomes, hybrid fertility and from isoenzymatic variability. Data concerning the ITS-2 intraspecific variation as well as the ITS-2 secondary structure are also presented.

09-069

PRIMARY AND SECONDARY STRUCTURE OF ITS2-rDNA IN *CULEX PIPIENS* L. AND IN *CULEX QUINQUEFASCIATUS* SAY.

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DNA sequences including the second internal transcribed spacer (ITS2) and some flanking 5.8S and 28S ribosomal RNA coding regions were compared within the two sibling mosquito species *Culex pipiens* L. and *Culex quinquefasciatus* Say. For correct species identification, male terminalia were identified by the DV/D ratio. Sequences were determined from polymerase chain reaction amplified DNA from seven field populations of both species (three populations of *Culex pipiens* from Italy, three populations of *Culex quinquefasciatus* from Africa and one from North America).

The consensus sequences showed a 97% identity and identical G+C content; in fact the only differences observed are 6 mismatches (3 transitions/3 transversion), 6 single-base and 1 triple-base deletions/insertions. Secondary structures of ITS2-rDNA from both species were calculated with the FOLD program and drawn with SQUIGGLES program of Genetics Computer Groups (GCG version 7.0); the observed ITS2 secondary structures were very similar and presented areas of homology with three putative U3 snRNA boxes near the juncture of the spacer with the coding regions. The intraspecific variation was studied and the possibility that the two mosquito species may be distinct by restriction sites analysis is discussed.

09-070

FIRST PHYSICAL MAP OF THE MALARIA VECTOR *ANOPHELES GAMBIAE* REVEALS NON-RANDOM DISTRIBUTION OF CODING REGIONS

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Random cDNA clones, cosmid clones and RAPD polymorphic fragments have been localized by *in situ* hybridization to the ovarian nurse cell polytene chromosomes of the malaria vector *Anopheles gambiae*. We thus established 86 molecular markers for 110 sites within the whole *An. gambiae* polytene chromosome complement. The cDNA clones analysed were isolated at random and their exact localizations were determined by *in situ* hybridization. For fifteen of the cDNA clones, a partial nucleotide sequence has been obtained; and for nine of them, sequence searches in the GenBank database revealed high degrees of similarity with published sequences. The cosmid clones analysed were obtained as the result of screening with a few of the aforementioned cDNA clones of particular interest, or taken from a small set of randomly isolated cosmid clones. The RAPD clones are polymorphic fragments, potentially diagnostic for the various chromosomal forms of *An. gambiae* s.s. that are currently being analysed. Of particular interest is the non-random distribution of the cDNA clones on the three *An. gambiae* polytene chromosomes. The possible evolutionary implications of such an observation are presented with reference to the non-random distribution of paracentric inversions.

09-072

COMBINED MAPPING OF EST, TAS, RAPD AND PHENOTYPIC MARKERS IN *B. MORI* (LEPIDOPTERA:BOMBYCIDAE)Y. Yasukochi, N. Komoto, Y. Takasu, T. Kanda, T. Tamura
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Genome maps are one of the most important tools for molecular biology and genetics. To construct such a map in the domesticated silkworm, we have screened RAPD markers using 100 10-mer arbitrary primers. In combination of them, we have found more than 500 RAPDs. Moreover, we isolated telomere associated sequences (TAS) in order to use them as a landmark specific for chromosome end region. Using a ligation mediated PCR method, 162 TAS were cloned and sequenced. The resulting sequences were utilized to establish sequence tagged sites. Known sequences derived from DNA data bases were also converted to expressed sequenced tags (ESTs).

We performed linkage analyses with landmarks described above and several phenotypic markers. As a result, we have constructed a genetic map containing 28 independent linkage groups corresponding to 27 autosomes and X chromosome of the silkworm.

09-071

PHYSICAL MAPPING IN AN ARTHROPOD VECTOR OF ANIMAL DISEASES: *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE) AND THE BLUETONGUE VIRUSESR.A. Nunamaker, S.E. Brown¹ and D.L. Knudson¹Arthropod-borne Animal Diseases Research Laboratory, USDA,ARS, Laramie, WY USA - ¹ Department of Entomology, Colorado State University, Fort Collins, CO USA

A recombinant cosmid library is being used as probes in FISH physical mapping of *Culicoides variipennis*, the primary vector of bluetongue virus in North America. Metaphase chromosomes were prepared from a *C. variipennis* cell line (KC). Probes were nick-translated with biotin-11-deoxyuridine triphosphate using standard protocols. To eliminate the confounding effects of KC DNA repetitive sequences, suppression hybridization with a KC Cot-1 DNA fraction was necessary. The specific hybridization was detected with avidin-fluorescein isothiocyanate (FITC). Slides were counterstained with diamidino-2-phenylindole (DAPI). The DAPI- and FITC-stained digital images were captured with a cooled-array, charge-coupled device collector mounted on a Zeiss Axioskop microscope. Digital images were enhanced, colorized, and merged, and chromosomal lengths and features were measured. A physical map of the *Culicoides* genome will be constructed to enable mapping of loci that control vector competence for bluetongue virus. Specific probes for vector competence loci will allow monitoring of vector populations and assessment of their potential as vectors for bluetongue virus and related, introduced pathogens.

09-073

ISOLATION OF CHROMOSOMAL INVERSION BREAKPOINTS IN THE *ANOPHELES GAMBIAE* COMPLEXK. D. Mathiopoulos, R. D. C. Saunders¹, A. della Torre, G. Mariotti, V. Predazzi², M. ColuzziIstituto di Parassitologia, Fondazione "Pasteur-Cenci-Bolognetti", Università "La Sapienza", Roma, Italy; ¹Department of Anatomy and Physiology, University of Dundee, Dundee, UK; ²Dipartimento di Genetica e Biologia Molecolare, Università "La Sapienza", Roma, Italy.

The six sibling species of the *An. gambiae* complex are characterized by fixed paracentric inversions on their chromosomes. The two principal malaria vectors of the complex, *An. gambiae* s.s. and *An. arabiensis*, are themselves subdivided into discrete units, carrying typical sets of polymorphic chromosomal inversions. Cloning both fixed and polymorphic inversion breakpoints is of particular interest since their DNA structure can provide unique information on naturally occurring inversions, point to a mechanism for their generation and maintenance in nature, as well as clarify the possible involvement of transposable elements. Furthermore, it should provide specific molecular probes for identification of the different inversion karyotypes.

Here we report the cloning of the first two breakpoints, the distal 2Rb of *An. gambiae* s.s. and the proximal 2Rd of *An. arabiensis*. For the 2Rb breakpoint, we used random clones from a library made from microdissected material from a breakpoint neighbourhood to localize, by *in situ* hybridization, the ones that lied closest to the breakpoint. These clones were then used as starting point for cosmid library screening and walking. One of the obtained cosmids seemingly spans the breakpoint, as judged by the two hybridization signals on the two breakpoints it gives in Fluorescent In Situ Hybridization (FISH) on polytene chromosomes of the inverted karyotype. Cloning of the 2Rd breakpoint of *An. arabiensis* was a result of screening of the cosmid library with a cDNA clone that cytologically mapped very close to that breakpoint. FISH of the cosmid gave three signals on the 2Rd/+ heterokaryotype, as expected. The two cosmid clones are being further characterized.

09-074

DNA FINGERPRINTING OF *BOMBYX MORI* L.: THE PROBLEM OF IDENTIFICATION OF INDIVIDUALS, OVIPOSITIONS AND BREEDS (LEPIDOPTERA: BOMBYCIDAE)G.A.Sevastyanova, A.P.Tretjak, A.P.Ryskov¹, Ju.B.Filippovich.

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DNA fingerprinting based on M13 phage DNA as a hybridization probe was used to analyse individual, oviposition and breed DNA's of *Bombyx mori* L. Each oviposition and breed DNA consisted of the mixture of hundreds and thousands individual genomes consequently. Nevertheless, similar, although not identical distribution of the hybridization bands in individual, oviposition and breed DNA's was found. The hybridization bands specific for breed fingerprints are also seen on individual and oviposition fingerprints. On the other hand, individual-specific hybridization bands are sometimes absent from oviposition and breeds fingerprints, due to a phenomenon of "individual genome dilution". Four most frequently observed hybridization bands characteristic of different breeds of *Bombyx mori* L. were designated as specific population markers. We assume that DNA fingerprinting can be useful of identification of *Bombyx mori* L. breeds.

09-076

MOLECULAR ANALYSIS OF OVERWINTERING DIAPAUSE IN THE SPRUCE BUDWORM

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The Spruce budworm, *Choristoneura fumiferana* exhibits an obligatory overwintering diapause as a second instar larva. The role of proteins and carbohydrates associated with this overwintering diapause was investigated. This insect uses both trehalose and glycerol as antifreeze agents to survive the winter. Trehalose levels in the hemolymph begin to increase as soon as they molt into the second instar and reach maximum within a week. This high level of trehalose is maintained throughout the 30 weeks of diapause. Glycerol levels on the other hand start to increase only after the second instar is exposed to cold conditions for four weeks and reach maximum by 10 weeks into diapause. The high levels of glycerol are maintained throughout diapause. Within three days after termination of diapause both trehalose and glycerol decrease to minimum levels. *C. fumiferana* larva also produces large quantities of two hexameric proteins (sub unit size 72 and 74 kDa) that are diapause related. These proteins accumulate in the first instar larval hemolymph beginning at four days after emergence and reach maximum levels by seven days after emergence. High levels of these proteins are maintained throughout diapause. The mRNAs (2.4 kb) coding for these proteins were present in large quantities during all seven days of the first instar. The mRNAs however decreased to undetectable levels as soon as the larvae molted to the second instar and entered the diapause. The mRNAs reappear in the middle of the last larval instar for 2-3 days before they disappear again. We have used differential display of mRNAs technique to identify genes whose mRNAs either increase or decrease during overwintering diapause. One of them is an insect homologue of Tcp20, a subunit of the eukaryotic TRiC chaperonin cloned from humans and yeast. One hundred and seventy six out of 331 amino acids are identical between human and *C. fumiferana* Tcp20. Supported by Canadian Forest Service and Science and Technology Opportunities Fund.

09-075

MOLECULAR GENETIC TECHNIQUES FOR DIFFERENTIATING CENTRAL EUROPEAN *ORINOCARABUS* SPECIES (COLEOPTERA, CARABIDAE)

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Due to a relatively high number of species, subspecies, micro-races and hybrids within the genus *Carabus*, there are many different opinions about subgeneric and subspecific classification.

In the present study we use molecular genetic techniques for taxonomic differentiation. Infra- and interspecific classification was inferred from SSCP (Single-strand conformation polymorphisms) analysis and RAPD-PCR (Random amplified polymorphic DNA) pattern based on the genomic Ubiquitin gene, which occurs both as single copy unit and in tandem repeats.

Investigations of mitochondrial NADH-Dehydrogenase (subunit 1) gene, which should infer phylogenetic relationships using sequence data, were also started.

09-077

GENOMIC INSTABILITY IN THE MEDFLY *CERATITIS CAPITATA*: AN EFFECT OF TRANSPOSABLE ELEMENT ACTIVITY?A.R. Malacrida¹, C. Torti¹, S. Costa¹, G. Gasperi^{1,2}¹Department of Animal Biology, University of Pavia, Pavia, Italy²Institute of Zoology, University of Sassari, Sassari, Sardinia, Italy

Our long term goal is to identify naturally occurring functional transposable elements in the geographic populations of the medfly *Ceratitis capitata*. To this aim we are analysing the genomic instabilities recurrently observed in the medfly. These instabilities are manifested by germline and somatic abnormalities. In particular our focus is centred on: 1) analysis of the hybrid dysgenesis syndrome recovered in the medfly (Torti et al., 1994, *J. Heredity* 85: 92-99), which resembles that of *Drosophila* where it is caused by the mobilisation of functional copies of transposable elements. The analysis of the dysgenic traits recovered in different medfly hybrids obtained from different crosses supports the hypothesis that more than one system is activated in the considered crosses. 2) The analysis of unstable mutations with particular attention to *white eye*. The *white eye* mutation of *C. capitata* affects, as in *Drosophila*, the pigmentation of larval and adult Malpighian tubules and the adult eye. It has been found to be localised on chromosome 5, on which most of the genes homologous to the X linked genes of *D. melanogaster* are located.

Genetic instability at the *white eye* locus has been observed in dysgenic crosses and in a *white eye* revertant line; it affects both the germline and the somatic cells. In the revertant line the germinal mutation rate of w^+ to w is 5.2×10^{-4} .

Preliminary Southern blot analyses on *white eye* and revertant w^+ flies indicate the presence of an insertion in the *white eye* locus. The nature of this insertion is now under investigation.

09-078

THE POTENTIAL OF GENE TARGETING FOR MANIPULATION OF THE MOSQUITO GENOME

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The exploitation of transgenic mosquitoes requires methods for the introduction of DNA both into living insects and into cultured cells. Ideally, this will involve a transformation vector which is capable of directing efficient and stable integration into the chromosomes of the recipient. Previous approaches have relied on transposition mediated mechanisms, principally those involving the P element from *Drosophila melanogaster*. However, the few reported cases of mosquito transformation show no evidence for the involvement of transposition, and there are currently no other transposable sequences (either from the mosquito or elsewhere) which have been developed into efficient transformation systems. We have been investigating the potential for gene targeting in mosquitoes through homologous recombination between donor and recipient sequences. A variety of constructs have been introduced into cultured cells derived from both *Aedes aegypti* (Mos20) and *Anopheles gambiae* (Ag55). Initially, this work focused on the optimisation of selectable markers for the identification of transformed cells, and a number of stably transformed cell lines have been generated in both species. We have also investigated negative selection strategies for the enrichment of targeted events, by exploiting the sensitivity to gancyclovir of cells expressing the HSV-*tk* gene. More recently, we have investigated the behaviour of a variety of targeting sequences in these constructs, and their ability to direct targeted integration in mosquitoes.

09-080

RIBOSOMAL GENES AND INSERTION ELEMENTS WITHIN AND BETWEEN *APHIDIUS* NEES (HYMENOPTERA, BRACONIDAE) SPECIES

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The ribosomal gene structure of a set of closely related Aphidiinae species belonging to the genus *Aphidius* Nees, of relevant interest in biological control, has been analyzed. *Aphidius ervi* genomic libraries have been constructed, several rDNA repeating units have been cloned and characterized and different regions of the rDNA cistrons have been sequenced. Insertion elements interrupting the *A. ervi* 28S rDNA genes have been identified and sequenced. The sequence of the two 5' and 3' insertion-28S junctions shows that the elements are inserted in the 28S gene at exactly the same position where R1 elements are present in *Drosophila melanogaster*, *D. virilis*, *Bombyx mori* and other insect species. In addition, also in the *A. ervi* rDNA, the insertion of the element produces a duplication of the 14 nt target region. The various *A. ervi* rDNA cloned regions have been used in Southern blot experiments with genomic DNA of closely related Aphidiinae species belonging to the genus *Aphidius*. The data will be presented and discussed in relation to the origin, function and evolution of rDNA insertions within these insect species.

09-079

TELOMERIC REPEAT ASSOCIATED RETROPOSONS IN THE SILKWORM *BOMBYX MORI*

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Telomeres of the chromosomes of a lepidopteran insect, *Bombyx mori* consist of pentamer-repeat sequence, (TTAGG)_n. This sequence is conserved in most insects except for dipteran and several other orders. A dipteran insect, *Drosophila melanogaster*, has no such short repeat at chromosome ends, but has retroposons (HeT-A and TART) instead. To study the general mechanism of telomere formation in insects, we analyzed the telomeric structure of *Bombyx* chromosomes. Within the telomeric repeat of *B. mori*, we have identified three different retroposons (TRAS1, 2 and 3). FISH has demonstrated that TRAS1 and TRAS2 are clustered at the tips of chromosomes of *B. mori*. All of these retroposons have the ORFs which are homologous to reverse transcriptase (RT) domain of non-LTR retroposons. The amino acid sequences of the RT domains of the three retroposons fall into one lineage with a retroposon R1 which is identified in rDNA of most insects. TRAS1, 2 and 3 are novel families of non-LTR retroposons which are inserted into the telomeric repetitive sequences as target sites.

Section 10

Ecology and Population Dynamics

10-001

THERMAL SENSITIVITY AND POPULATION RESPONSES TO CLIMATE CHANGE

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Holometabolous insects perform quite different tasks during their larval and adult stages. As a result, the effects of temperature on fitness components in larvae (e.g. larval survival, feeding and growth rates, development time) and adults (e.g. flight, mating, oviposition) may differ substantially. I will combine evidence about thermal sensitivities for different life stages with simple ecological models to explore how climatic variation may alter larval and adult fitness components, and their consequences for demography and growth rates of temperate insect populations.

10-002

THE POPULATION DYNAMICS AND CONSERVATION OF A RARE BUTTERFLY: THE CASE MODEL OF *MACULINEA REBELI* (HIR.) (LEPIDOPTERA: LYCAENIDAE)

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Caterpillars of the butterfly, *Maculinea rebeli*, feed sequentially in flower-buds of *Gentiana cruciata* and in nests of *Myrmica schencki* ants, with which they have a sort of 'cuckoo bird' relationship. Ants feed them in preference to their own larvae, reducing the production of new workers and hence the colony size the following year. Other species of *Myrmica* compete with *M. schencki* for nest-sites. They adopt caterpillars with equal facility, but fail to rear them to maturity and are consequently less damaged.

Using a mathematical model we explore how species interactions can influence the populations of the butterfly, *M. schencki*, and other *Myrmica* species.

Addition of the butterfly to the system reduces dramatically both the number and average size of *M. schencki* nests. As a result, the number of nests of other *Myrmica* increases as they encroach onto the drier areas. In intermediate areas, where most competition occurs between the ants, the nest-size of other *Myrmica* increases, but overall, their average size also falls because of the direct effect of the caterpillars.

From a conservation stand-point, we suggest that a subtle combination of changes in the environment can either benefit or endanger the butterfly population depending on how each of the ant species are affected.

10-003

BUTTERFLY MOVEMENTS, METAPOPOPULATIONS AND CONSERVATION

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Large numbers of butterfly species have severely declined or have become already extinct in many countries in especially northern Europe. Habitat destruction and fragmentation appear to be the main causes of extinctions in most species. Increasing numbers of species now occur as metapopulations, consisting of extinction-prone local populations. The persistence of such species depends critically on movement behaviour and factors affecting it. Butterflies range from very sedentary species to very mobile species, with important implications for the consequences of habitat fragmentation on their dynamics. Density-dependent and patch size-dependent emigration and immigration rates increase the extinction risk of small populations, and may create an Allee effect in local population dynamics. Substantial movement among small populations in a metapopulation may lead to a rescue effect and multiple equilibria in metapopulation dynamics. Such complex spatial dynamics may lead to a rapid and catastrophic extinction of species from a large region without any or substantial change in the environment. One important question of which little is presently known is how fast the movement behaviour may evolve in butterflies in response to a changing environment.

10-004

DYNAMIC MOSQUITO BEHAVIOUR: IMPLICATIONS FOR MALARIA EPIDEMIOLOGY

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Recent work on theories of malaria epidemiology conclude that deviations from constant biting terms can have profound effects on infection rates. What is not clear, however, is what form the biting terms should take. To remedy this, we employ concepts from Behavioral Ecology to develop a dynamic life history model that predicts changes in blood and sugar host preference as a function of several factors, including: 3 physiological states (i) crop nectar volume and concentration; (ii) somatic energy; and (iii) age; and two ecological states; (iv) nectar availability; and (v) blood availability. Explicit constraints are: (vi) maximum crop volume; (vii) maximum midgut volume; (viii) nectar processing rates; and (ix) life expectancy.

We use the dynamic model to elucidate the population distribution of Host Attack Thresholds under different ecological conditions. We then show how one can use this distribution along with descriptors of Host Quality, Host Defense Thresholds and Vector Attack Persistence to develop a realistic biting term. From there, we explore the impact of such complex terms on infection rates.

Our talk is concept-dense and detail-light and is meant to demonstrate how an evolutionary approach can be used to enhance our understanding of population-level phenomena.

10-006

EGGLOAD DYNAMICS, FORAGING EFFORT AND OVIPOSITION RATE IN A PARASITIC WASP

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Estimating the oviposition rate in the field is a very difficult yet important problem in insect population dynamics. Two approaches are available: a direct approach, observing foraging females in the field; an indirect approach, using the dynamics of the number of mature eggs carried by a female at any moment in time, called the eggload. The second approach is used here to estimate the rate of oviposition of the parasitoid *Aphytis melinus* (Hym. Encyrtidae), the biological control agent of the California red-scale on citrus. The eggload at different points in time and the rate of egg maturation are known from field and laboratory experiments. We model the dynamics of the eggload using a family of queuing models, which includes the simplest birth-death model and much more complex ones where the rate of oviposition is a function of the eggload and with an upper limit for the storage of mature eggs. The eggload does not reach a steady state over the period of one day. This implies that a careful choice of the characteristics of the eggload distribution must be made when testing the models, as the bulk of the distribution does not behave dynamically as the tails do. These models enable us to predict how often and when *Aphytis* runs out of eggs during the foraging period, and, if so, what the chances are of generating and laying more eggs during the same day and to what extent the upper capacity of storage is limiting. Finally, we compare the rate of oviposition obtained by this modeling approach with that obtained from direct observations of females foraging in the field.

10-005

DYNAMICAL EFFECTS OF PARASITOID ATTACKS DEPENDENT ON HOST SIZE AND PARASITOID STATE

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Some parasitoids mature new eggs by feeding on (and killing) hosts. On encountering a host individual, such parasitoids may reject the host, feed on it, or parasitize it and, if the last, may lay only 1 or multiple eggs, which may be either male or female or a mixture. The actual outcome depends on both properties of the host, especially size in many instances, and also the state of the parasitoid, especially its egg load. We have developed host-size-and parasitoid-state-structured models that explore the population dynamics consequences of such decisions. We show that several apparently disparate aspects of parasitoid behavior in fact have the same dynamical outcome because they are actually different manifestations of a broader phenomenon. We also show that the competitive displacement of one *Aphytis* parasitoid by another species of this genus, which led also to improved control of Californian red scale, can be explained by rather subtle differences in size-dependent behavior between the two species. Parasitoid behavior can thus be shown to have potentially important consequences for the stability of parasitoid-host interactions and for biological control of insect pests.

10-007

DO PARASITIC WASPS AFFECT THE SPATIAL RESOURCE USE OF THEIR HERBIVOROUS HOSTS? AN EVOLUTIONARY GAME BETWEEN ROSE HIP FLIES AND THEIR PARASITOIDS

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Rose hip flies (*Rhagoletis basiola*, Diptera: Tephritidae) visit clusters of rose hips, where they lay single eggs beneath the skin of the fruits. There, fly eggs are vulnerable to the attack of a specialized egg-larval parasitoid (*Halticoptera rosae*, Hym.: Pteromalidae). Female flies were found not to oviposit into every fruit of a cluster, but to spread their eggs out across clusters. In turn, parasitoid females were found to be more effective in the search for hosts on heavily infested compared to mildly infested clusters. We used theoretical models to elucidate how competition between flies, habitat variability, and the threat of parasitoid attack affect reaction norms for the spatial distribution of fly eggs across rose hip clusters. We investigated different scenarios, with evolutionary games between flies only, between flies and a constant threat of parasitoids, and evolutionary games where reaction norms of flies and parasitoids could coevolve.

10-008

CARABID POPULATION AND COMMUNITY FEATURES AS AN "ADAPTATION" TO THE LANDSCAPE SYSTEM: IMPORTANCE OF THE ECOTOPE AS LANDSCAPE UNIT.

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The landscape system contains smaller landscape units called ecotopes by landscape ecologists. The most useful definition of ecotope refers, in our experience, to well delimited geomorphic units, like rivers, lakes or other riverside habitats, mountain slopes belonging to homogeneous geologic formations, etc.

The authors compared the Carabid beetle groupings of two landscapes: the Northern Adriatic system (with about 400 species censused and 7 ecotope-types sampled) and the Mediterranean system of Calabria (8 ecotopes, 180 species).

The ground beetles living in a single ecotope may be more or less numerous, (differences in diversity values); interesting differences are observed also in: reproduction rhythm (summer larvae versus winter larvae); dispersal power (high versus low percentages of flying species or individuals); population densities; life tactics; population distribution and migration within the ecotope; choice of hibernation sites; hygrophily and resistance to desiccation, etc.

The coenoses assemblages within an ecotope are strictly interrelated through ecological succession processes, but, on the contrary, exchanges from one ecotope to the neighbouring one are often extremely weak, depending especially on differences of geomorphology and subsoil-water level.

On the whole, two extremes could be recognized: **zonal ecotopes**, where the climax ecosystem is easily reached, and carabid populations and communities show more sedentary features, and **azonal ecotopes**, where the beetles (and the other animals) are living in a more instable situation, with more opportunistic, discontinuous or temporary populations. The adaptive "space" of many species seems to be shaped on the habitat complex of an ecotope, more than on the conditions of a single habitat type. Ecotopes could be used in animal cartography.

10-010

STRONG INTERCONNECTION BETWEEN ADAPTIVE TRAITS AND POPULATION STABILITY IN AN HERBIVOROUS LADY BEETLE

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An herbivorous lady beetle *Epilachna niponica* exhibited a high stability of population densities in terms of host plant abundance. Field experiments revealed that reproductive females resorbed eggs in the ovary in response to host plant deterioration. Also, females selected oviposition site carefully, by laying eggs on plants with less egg load. These specified oviposition tactics resulted in a temporal resource tracking of populations, through density-dependent reduction in realized eggs per female. Lifetime fitness of offspring decreased with increasing egg density. This implies that oviposition on plants with less egg density improves lifetime reproductive success of an ovipositing female. Egg resorption could have adaptive significance when offspring fitness drops considerably due to resource deterioration late in the reproductive season or habitat disturbance such as inundation. It also enhances a chance of future oviposition in the second reproductive season, through increasing subsequent survival of egg-resorbed females. Consequently, the oviposition behavior of reproductive females in time and space is highly correlated with lifetime fitness of their offspring.

10-009

CHANGING INTERPRETATIONS OF ADAPTATIONS INFLUENCE VIEWS ON POPULATION DYNAMICS

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The most widely accepted view of evolutionary change is that of ongoing adaptive change in all populations. The development of ecological and evolutionary theory is thus confounded by general acceptance, either tacitly or explicitly, that species are adaptive devices. The alternative view disassociates the process of speciation from its ecological consequences. The two views contrast sharply on how speciation occurs. In the former, isolationist view of species, speciation is a continuous process driven ultimately by competition. Consistent with the isolationist view of species is the body of population ecology theory that emphasises demographic parameters. The alternative, recognition, view of species is more positive because it emphasises adaptations of the fertilisation system. Here, speciation is an incidental consequence of a change in the fertilization system. Such change is constrained to take place only in small isolated populations undergoing directional selection in a habitat to which the species is not currently adapted. The recognition view of species is inconsistent with changing abundance being driven by demographic or population features. Rather, the dynamic interaction between individual and environment is given special prominence. The two views therefore have different implications for the study of population ecology. The former, when not idealistic is merely descriptive, with low predictive power. The latter is predictive once the basic biology, adaptations and environmental requirements of the species are defined through adequately designed observations and experiments.

10-011

ON THE SURVIVAL VALUE OF WING DIMORPHISM IN CARABID BEETLES

B. Aukema (Wageningen-The Netherland)

ABSTRACT NOT RECEIVED

10-012

THE RELEVANCE OF LIFE HISTORY THEORY FOR
CARABID SPECIES OF WESTERN EUROPE
P. Den Boer (Wijster-The Netherlands)

ABSTRACT NOT RECEIVED

10-014

ANCIENT POPULATIONS IN ANCIENT WOODS: DISPERSAL,
AGE STRUCTURE, AND VARIABILITY OF *CARABUS GLABRATUS*
PAYKULL IN NORTHWEST-GERMANY (COLEOPTERA:
CARABIDAE)

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Woodlands were extensively destroyed by man in Northwest-Germany during Middle Age and early Modern Times. But not all of them were cleared away; those belonging to aristocracy or to ecclesiastical institutions were preserved. Since about 1800 modern forestry has been established entailing reforestation on a large scale. According to their age, woods can be divided in ancient and recent ones. Some Ground Beetles show a relict distribution in ancient stands, whereas other Carabids have spread widely and occur also in recent woods.

Patterns of movement of the relict species *Carabus glabratus* were studied by harmonic radar and, in an circular enclosure, with capture-recapture experiments of individually marked beetles. Locomotory activity is lower in this species than in *Carabus problematicus* living in ancient and recent woods.

Allozyme studies of esterases and the phosphogluconate dehydrogenase in 20 populations show a common genetic variability with up to 7 allozymes (EST-X) per population. Therefore, long lasting extreme bottlenecks resulting in the loss of genetic variability must be excluded for the studied populations.

The populations are composed of young and two or more year old individuals, as revealed by the presence or absence of the Corpus luteum before the reproduction period, and by mark-recapture experiments in an enclosure. The possible role of old individuals in preventing strong population dynamics is discussed.

10-013

THE DEVELOPMENT OF THE AGE PYRAMID IN A LOCAL
POPULATION OF *CARABUS AURONITENS* FABRICIUS
(COLEOPTERA, CARABIDAE)

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In the Westphalian Lowlands *Carabus auronitens* is a spring breeder. Preimaginal stages develop during 3-4 months in summer. Young beetles emerge asynchronously in late summer/early autumn. They are obligatorily active for food during 3-5 weeks. Surviving old beetles are dormant from the end of the spring season until the beginning of the next spring season. During the spring season adults of at least 3 generations are active. We measure the rate of yearly net reproduction and the rates of interseasonal age-dependent mortality of a local population fenced in a beetle-proof enclosure (0.2 ha). We analyse the dependence of the surviving probability of individually marked adults on (1) time of hatching in the late summer/early autumn season, (2) body size, (3) weight development, (4) level of locomotor activity and (5) start and end of the individual season in spring.

10-015

STABILIZATION OF A *LAEMOSTENUS SCHREIBERSI* POPULATION
(CARABIDAE) AS AN EFFECT OF ADULT LONGEVITY - RESULTS OF A
LONG-TERM INVESTIGATION OVER 10 YEARS.

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A population of the microphthalmic carabid *Laemostenus schreibersi* was studied in a cave near Villach in Carinthia (Austria). Results of a long-term investigation over ten years (1986 - 1995) are presented. During 15 seasons of field observations the beetles were trapped in pitfalls, individually marked and released inside the cave. Using the mark-recapture method it was possible to estimate population size and the patterns of locomotion activity.

Other aspects of population dynamics are concerned with seasonal and spatial distribution in the different cave-compartments, sex-ratio, reproduction and development.

The portion of recaptured beetles was very high (62 %). The rate of exchange with other populations living in the fissure-system was evaluated. Population size is small, but rather constant (50 - 110 individuals). The beetles of *Laemostenus schreibersi* can reach the remarkable age of at least 7.5 - 8 years. Longevity is discussed as a factor stabilizing population size. The survival time of adult beetles throughout some years is known from several carabid species, but such an extreme longevity has not been reported before. Reproduction-rate seems to be rather low.

10-016

Cooperation within Europe in a Study of *Pterostichus oblongopunctatus* F. (Coleoptera, Carabidae) - Survival and Reproduction.

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Abstract

Observations were made of the carabid fauna in more than dozen forest stands in Germany, Holland and Poland. Special attention was paid to *P. oblongopunctatus*. Wherever possible the following were estimated for this species: the male-to-female ratio; individual biomass at the moment of capture, after 24 h. of starving and after 24 h. of feeding; respiration; consumption; number of eggs laid; number of eggs in ovaries; age structure of adults and the period of activity. Differences in the characteristics studied were found between groups of individuals from different stands frequently there were differences between neighbouring stands, and these were more pronounced than those between stands some hundred kilometres apart. It is suggested that, with successional changes of habitat *P. oblongopunctatus* changes its life history pattern by having larger body dimension, lower respiration and consumption, a shorter lifespan of the adults, a larger number of eggs in the ovaries and different activity of males and females giving rise to a difference in the sex ratio in pitfall traps. The supposition is advanced that the main factor governing the way of life strategy is food. The food conditions for larvae and adults respectively may be estimated by the dimensions of the adult and the ratio of males to females in pitfall traps.

10-018

PROLONGED DIAPAUSE AND POPULATION DYNAMICS OF THE NOBLE FIR SEED CHALCID *MEGASTIGMUS PINUS* PARFITT (HYMENOPTERA: CHALCIDOIDEA)

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Over a seven year period the interactions between Noble Fir (*Abies procera*) and the seed chalcid *Megastigmus pinus* were investigated. Imagines seed chalcid population size was estimated from emergence traps placed on the forest floor and overwintering larval population size from cone production and infection rates. Furthermore, imagines were reared each year from freshly collected cones which were kept in emergence boxes in the forest.

Megastigmus pinus larvae were shown to overwinter two-three years thus entering prolonged diapause. In years of extremely low cone production prolonged diapause was high, i.e. few imagines emerged from cones of the preceding year. In years of abundant cone production almost no larvae of the preceding year entered prolonged diapause and thus emerged as imagines that year. In years of moderate cone production prolonged diapause varied considerably between trees.

The pattern of prolonged diapause exerted a moderating effect on the population dynamics of the seed chalcid. In the study period, population densities of emerging imagines varied only 7-fold, whereas cone production varied 100-fold. This leads to high infection rates in low seed years and low infection rates in years of high cone production.

10-017

LIFE CYCLE EVOLUTION OF CARABID BEETLES IN GALÁPAGOS: A LONG TERM SAMPLING STUDY

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As part of a long term study on terrestrial arthropods (especially spiders and carabid beetles) and in cooperation with Austrian, Canadian and Ecuadorian scientists, we have been able during recent years to sample the most important islands and volcanoes of Galápagos in complete transects or in altitudinal gradients. The carabid faunal diversity is now estimated at some 40 species, the majority of these being endemic to the Galápagos Archipelago or even to single islands or volcanoes. In previous papers we have addressed already several questions related to the biogeography, evolutionary ecology and speciation of some of these beetles in Galápagos.

In this contribution we present the first results of a long term sampling study, conducted by continuous pitfall trapping in several habitats of Isla Santa Cruz during more than two complete year cycles (1991-1993; in cooperation with the Charles Darwin Research Station). This monitoring project aimed at understanding additional aspects of the biology of this intriguing fauna, in particular with regard to population dynamics and life cycle timing and evolution in the extreme circumstances of Galápagos. Such studies on invertebrates have, to our knowledge, never been performed before in Galápagos. These data also extend our current knowledge on dispersal power and habitat preference of particular species. Both are important aspects in the study of adaptive radiation.

The life cycle of the most abundant carabids is reconstructed by analyzing the seasonal occurrence (phenology) of larvae and adults and by unravelling the population structure. Dissection of beetles enables to ascertain their reproductive state and yields information on the age composition of the population. Finally an attempt is made to relate the timing and duration of the life cycle to possible environmental (climatological) or biotic (presence of prey species) cues and these results are discussed in an evolutionary context.

10-019

CAN LOCAL ADAPTATIONS EMERGE IN ISOLATED POPULATIONS OF THE WATERSTRIDER *LIMNOPORUS NAJAS* (HETEROPTERA GERRIDAE)?

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Local adaptations are suggested to benefit individuals in the local conditions under which they evolved. In isolated populations, however, the homozygosity caused by bottlenecks and genetic drift may reduce the genetic variance available for selection and may therefore prevent the evolution of such adaptations. Furthermore, the relatively short time elapsed since the last glaciation in Northern Europe has limited the possibility of local adaptations to emerge.

We have experimentally studied the possibility of appearance of local adaptations in the overwintering behavior of *Limnopus najas* in ten isolated populations. The species overwinters in streamsides as an adult and copulation begins in the spring. In Southern Finland winter is several weeks shorter than in Central Finland. To test if populations differ in their overwintering behavior we transferred five populations from Southern Finland and five populations from Central Finland to five rivers in Central Finland in which there are no natural populations of *L. najas*. The time taken to begin overwintering in each of the five rivers by each pair of populations was subsequently measured.

Preliminary data obtained suggests that there may be differences in the overwintering behavior of some of the populations. The reasons behind such differences are discussed.

10-020

POPULATION GENETICS AND DYNAMICS OF
TRANSGENIC ARTHROPOD NATURAL ENEMIES: AN
OVERVIEW OF POTENTIAL RISKS AND LOGISTICAL
ISSUES

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The development and deployment of genetically-manipulated arthropod natural enemies has, to date, involved predators and parasitoids that have been altered by traditional breeding methods. The use of recombinant DNA methods should improve the efficiency of developing new strains but will require careful consideration of environmental risks and approval of various regulatory agencies.

An overview of previous genetic improvement programs will be provided with discussion of the gaps that were discovered in our knowledge of the population genetics and biology of the target species involved, including the predatory mite *Metaseiulus occidentalis*, the predator *Chrysoperla carnea*, and the parasitoids *Trioxys pallidus* and *Aphytis melinus*. The deployment of transgenic arthropods for practical pest management programs will require the collaboration of molecular biologists, population ecologists and geneticists as well as entomologists with an understanding of the logistical difficulties of large scale pest management programs. Several issues remain to be resolved regarding appropriate risk assessment methodologies.

10-022

THE POPULATION BIOLOGY OF CYTOPLASMIC
INCOMPATIBILITY: DATA AND THEORY

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In many insect species, cytoplasmically transmitted microbes related to *Wolbachia pipientis* cause reduced egg hatch when infected males mate with uninfected females. This "cytoplasmic incompatibility" (CI) tends to confer a reproductive advantage on infected females in polymorphic populations, allowing the maternally transmitted, CI-inducing infections to spread within and among populations. In *Drosophila simulans*, a CI-inducing microbe has rapidly spread northward from southern California over the past decade. The intra-population dynamics of this spread and the equilibrium infection frequency can be understood in terms of a simple mathematical model with parameter values—describing maternal transmission and levels of incompatibility—estimated directly from natural populations. The infection's wave of advance has been accompanied by predicted changes in mtDNA haplotype frequencies. This infection is now widespread in *D. simulans* populations throughout North and South America and Europe, and it serves as a model for the possible manipulation of insect pests via CI. Both empirical and theoretical work on population dynamics will be reviewed, along with results concerning the population biology of multiple infections and coevolutionary changes in CI-inducing microbes and their hosts.

10-021

HORIZONTAL GENE TRANSFER IN POPULATION
DYNAMICS

D. Hartl (Harvard-United States)

ABSTRACT NOT RECEIVED

10-023

POPULATION GENETICS AND POPULATION DYNAMICS
MODELS: WHERE DO WE GO FROM HERE?

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Recently, the ecological theory of metapopulations has greatly advanced by focussing on structured models of metapopulations which include population sizes within patches. The population structure assumed in metapopulation models is appropriate for many terrestrial arthropod species. I will outline research directions which will focus on structured models of metapopulations which include allele frequencies as well. This work will thus focus on the joint action of ecological and genetic forces in maintaining diversity within a metapopulation. I will contrast these approaches with alternate approaches using cellular automata and reaction diffusion equations.

10-024

ANALYSIS OF THE GENETIC CONTROL PROGRAM FOR THE AUSTRALIAN SHEEP BLOWFLY, *LUCILIA CUPRINA* IN AUSTRALIA: A PARADIGM FOR RELEASES OF TRANSGENIC PEST ARTHROPODS?

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The Division of Entomology CSIRO, has developed methods to control the Australian Sheep Blowfly, *Lucilia cuprina* using genetical techniques. As one of the largest and most "mature" genetic control programs in existence, sharing the lessons that this species has taught us over the last 25 years may prove useful to those contemplating the use of transgenic insects to control pests. Some of the problems and gaps in our knowledge that impeded progress are species specific, while others are specific to the use of chromosomal rearrangements as a means of imparting genetic load to a natural population. Yet other difficulties encountered while attempting to implement genetic control strategies for *L. cuprina*, are likely to be more general, and thus may complicate the implementation of any attempt to impose genetic change on a natural population through the release of genetically modified insects.

10-025

MODELLING THE DYNAMICS OF AGE-STRUCTURED ARTHROPOD POPULATIONS

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The von Förster and the discrete Leslie analogous models are concise descriptions of the dynamics of age-structured populations, but have limited predictive capabilities. The exclusive use of chronological age has been found inadequate for modelling the population dynamics of poikilotherms. The quality of the model predictions is improved if physiological age in terms of developmental level (e.g. thermal units, size, biomass) is incorporated into temperature-dependent development. Since there is variation between the individuals in a population and in environmental factors, the model should embody a stochastic or random element; biological and environmental variability are described by means of stochastic differential equations. Two main approaches are possible: macroscopic models which describe gross changes in population development, microscopic models which describe the life history of individuals. The latter models appear to be particularly useful in pest management system and in population ecology. Simulation studies, with several models, are analyzed and discussed.

10-026

APPROACHES FOR MODELLING INSECT PHENOLOGY

S. Worner (Christchurch-New Zealand)

ABSTRACT NOT RECEIVED

10-027

EXTINCTION AND THE TEMPORAL VARIATION OF POPULATIONS

B. McArdle (Auckland-United States)

ABSTRACT NOT RECEIVED

10-028

QUANTITATIVE ANALYSIS OF GYPSY MOTH SPREAD IN THE CENTRAL APPALACHIANS

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Gypsy moth, *Lymantria dispar* (L.), was introduced into North America near Boston in 1869 and since that time it has been slowly expanding its range to the west and south. In attempt to slow its spread, USDA Forest Service has established several barrier zones in which isolated colonies are detected and eradicated. To evaluate the effect of barrier zones on the rate of gypsy moth, I suggested a new method for measuring the rate of spread. It was measured as the average distance between regular population boundaries (regular boundaries have no "islands", gaps or folds) in consecutive years. Population boundaries were estimated using male moth counts in pheromone traps, egg mass counts, and defoliation maps in the central Appalachian Mts. in 1988-1995.

The boundary of 1 moth/trap was on average 110 km from the boundary of defoliation, and male moth capture rate increased 10 times per 29 km perpendicular to the population front. Approximately 11 yr separated the time when traps caught 1 moth/trap until defoliation first occurred in the same area.

Gypsy moth spread rate declined from 1988 to 1995 by ca. 30-60%, as measured from 1) time series of spread rates and 2) boundary "compression" (reduction of the distance between adjacent boundaries). Reduction of gypsy moth spread rate may have been due to eradication of isolated colonies in the study area.

I developed a model of gypsy moth spread that considered establishment of isolated colonies beyond the moving population front due to inadvertent transportation of life stages on human vehicles. The probability of colony establishment decreases with increasing distance from the population front. The model predicts that the barrier zone in the Appalachian Mts. should reduce the rate of gypsy moth spread by ca. 50% which is consistent with our data.

10-030

Measurements of spatial pattern and spatial association for counts of insects

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A system will be described to measure the spatial pattern of counts of insects that has an improved intuitive basis, compared with traditional more abstract, mathematical approaches, and utilises fully all the available spatial information in the sample. Indices and tests of non-randomness will be described. An algorithm will be presented to simulate permutations of a given set of insect counts to achieve a new arrangement with a specified degree of aggregation. This is particularly useful in the generation of realistic data to compare several sampling strategies. A further algorithm finds a permutation of a given set of counts of one species over predefined locations, at each of which there is also a count of a second species, for a specified degree of association between the two. Various visual aids to the analysis of spatial pattern will be presented. The problem of measuring and testing for association for two sets of counts sampled at the same locations will be discussed and recent progress summarised. Fortran software is freely available from the author.

10-029

GEOSTATISTICAL METHODS FOR TESTING CORRELATIONS: APPLICATION TO LANDSCAPE LEVEL FOREST DATA

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Testing the statistical association among two or more variables is one of the most common approaches to evaluation of hypotheses in ecological research. Classically, researchers test these associations by collecting multiple samples of each variable replicated through space and calculate correlation coefficients. The assumption in this approach is that spatially stratified samples are independent. However in many (if not most) systems values may be spatially autocorrelated at the scale at which samples are separated. We present here a method for testing for correlations between two variables that are known to be autocorrelated. Sample variograms are first estimated from sample data in order to quantify spatial autocorrelation. Variograms and frequency distributions are then used in a sequential simulation procedure to generate multiple realizations of samples that honor the sample variogram and histogram but are not inherently correlated. The frequency distribution of simulated correlation coefficients are then used to test the statistical significance of the observed correlation coefficient.

10-031

IMPLICATIONS OF DIFFERENT TYPES OF ADAPTATION FOR INTERACTIONS IN FOOD WEBS

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A lack of any dynamic equations describing adaptation has been a failure of many previous dynamic models of food webs. Adaptation can occur by behavior, evolution, or shifts in the distributions of different age classes or phenotypes within a species. I use a series of simple models of two- and three-trophic-level communities to explore the implications of these different types of adaptation for food web behavior. The main behaviors investigated are how population densities respond to top-down and bottom-up environmental effects.

10-032

MECHANISMS AND PROPERTIES OF RATIO-DEPENDENT MULTITROPHIC MODELSAlan A. Berryman¹, R. Arditi², J. Michalski², X. Chen¹¹ Department of Entomology, Washington State University, Pullman WA USA - ² Institute of Zoology and Animal Ecology, University of Lausanne, 1015 Lausanne, Switzerland

The general structure and underlying mechanism of ratio-dependent (R-D) trophic models will be described and contrasted to those of traditional Lotka-Volterra models. Some static and dynamic properties of R-D models will be described and predictions of R-D theory will be compared to empirical observations on the biological control of insect pests.

10-033

TRITROPHIC MODELING: A QUEST FOR REALISM, WHOLENESS AND GENERALITY

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The term, tritrophic system, is used here to mean an autotroph-herbivore-carnivore relationship that may be a linear food chain or a food web of any degree of complexity. The question of how to model such interactions has provided grist for controversy since the mid 1920's. Theorists simplified the biology for mathematical tractability and biologists were often lost in minutia. The former sought high level theory and the latter practical solutions to real world problems. We now recognize that the use of mathematics to describe weak biology (or *vice-versa*) is unlikely to be achieved for population ecology. Holling's goals of realism, wholeness and generality -- i.e., unifying principles. Today the schism between theoretical and field ecologists is being breached and annealed via educated discussion. The notions of meta-organisms and meta-populations capture the details that concerned field ecologists. Increasingly models that include the behavior and physiology of resource acquisition and allocation are appearing in the literature, and field ecologists are examining the underlying properties of their models. In this paper, tritrophic models based on the supply - demand metabolic pool paradigm and the underlying theory are reviewed. A range of models including analytical models, numerical simulations of meta-organisms and object oriented models of meta-populations based on a supply-demand paradigm are reviewed and tested against field data.

10-034

UNDERSTANDING THE POPULATION DYNAMIC INTERACTIONS BETWEEN PARASITOIDS AND THEIR HOSTS

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Parasitoid wasps have proved excellent systems with which to investigate many aspects of predator-prey interactions and a rich theoretical literature on their population dynamics has developed. We review recent research in three areas. (i) The importance of spatial processes in influencing host-parasitoid dynamics. (ii) The population dynamics of communities of hosts and parasitoids. (iii) The application of host-parasitoid dynamics to specific systems, especially to answer questions raised in the design of biological control programmes, using "models of intermediate complexity". The review will illustrate the dual function of classical population dynamic modelling: to answer general questions about population dynamic behaviour, and to aid the interpretation of more complex models of individual interactions.

10-035

HOW COMMUNITY STRUCTURE INFLUENCES PREDATOR PERSISTENCE AND PREY REGULATION

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The effect of community structure and the interaction strengths between its constituents on persistence and regulation is examined using a model of multispecies interactions. The model employs a metaphysiological paradigm driven by a supply/demand functional response. Using it, we develop criteria for species persistence that address how various forms of competition and predation result in either coexistence, species displacement or multiple stable steady states. In all cases, relative ecological efficiencies between species that share prey determine these persistence criteria. We also examine two types of prey regulation. In the first case with only one prey species, we define regulation as the ratio of mean prey density with predation to mean density without predation. In the second case with two prey species exploiting a common resource, the ratio of mean resource density with prey to mean resource density with prey and predator determines regulation. In both instances, better regulation corresponds to a smaller value. Using these definitions, we determine what combination of predators regulates the prey most effectively and how it depends on the physiological and behavioral characteristics of the predator and prey species. For example, depending on relative values searching efficiencies between predators the introduction of an intraguild predator to a predator-prey system can improve or disrupt regulation by coexisting with or displacing its intraguild prey. Combining these analyses, we discuss why predator introductions may fail and therefore not effect regulation, succeed and improve regulation, or succeed and disrupt regulation.

10-036

APPLICATIONS OF MULTITROPHIC POPULATION MODELS TO FIELD PROBLEMS

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Insect pests occupy an intermediate trophic level and their abundance is affected both by bottom-up effects from the foodplant and by top-down effects from natural enemies. A tritrophic perspective is required to understand the dynamics of these interacting populations and to investigate the role that natural enemies play in the biological control of insect pests.

The application of tritrophic population models, based upon physiological determinants of resource acquisition and transfer between trophic levels, to biological control will be illustrated with reference to two pest systems. Firstly, a cotton-whitefly-aphelinid parasitoid system will be used to examine the influence of parasitoid reproductive strategies (primary parasitism, obligate autoparasitism and facultative autoparasitism) on the outcome of biological control. Assuming that sex allocation in autoparasitoids is dependent upon the relative abundance of parasitized and healthy whitefly nymphs, the model reveals the potentially disruptive nature of facultative hyperparasitism in biological control.

The second application of the tritrophic model examines whether the host stage attacked by a parasitoid influences the success of biological control in an alfalfa-aphid-aphidiid parasitoid system. Assuming that the per capita reproductive rate of an aphid is a linear function of the stage at which it is attacked, the model clearly shows the benefit to biological control of early parasitoid attack in the life cycle of the aphid.

10-038

THE ROLE OF PARASITOIDS IN STRUCTURING GRACILLARID LEAFMINER COMMUNITIES ON *CRATEGUS MONOGYNA* JACQ.

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It has been proposed that the occurrence of shared natural enemies between host species can limit the abundance of individual species within a community. This proposal is explored for the community of gracillarid leafminers (Lepidoptera: Gracillariidae) living on hawthorn (*Crategus monogyna*). Using data from eight leafminer generations, I examine the role of parasitoids in influencing leafminer population growth and in determining indirect interactions between leafminer species. The data suggest that host-specific parasitoids exert the largest effect on leafminer population growth, but shared parasitism, due to non-specific parasitoids, is significant in the dynamics of at least one leafminer species. The population growth of this species is influenced by the abundance of other leafminer species in the community.

10-037

PREDATORS, PARASITOIDS AND PATHOGENS AS MORTALITY AGENTS IN PHYTOPHAGOUS INSECT POPULATIONS

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Various ecological and biological factors influence the rate of enemy-induced mortality suffered by herbivorous insect populations. Life tables compiled for 82 holometabolous herbivore species were used to quantify such influences. Enemy-induced mortality by all three types as well as by each type separately were tested for differences associated with the developmental stage and five ecological characteristics of pre-adult herbivores (feeding biology, invasion status, and the successional stage, cultivation status, and latitudinal zone of the habitat). Total enemy-induced mortality is higher in the late developmental stages, and is heavier for exophytic than endophytic feeders; it does not differ with respect to any of the other four variables. Overall, parasitoids kill proportionately more victims than either predators or pathogens. The enemy-type X developmental stage interaction was not significant, indicating that increased attack by parasitoids as herbivores age is independent of predator or pathogen attack. However, enemy-type did interact significantly with four ecological variables: (1) predators and pathogens kill fewer endophytes than exophytes, whereas parasitoids are generally unaffected by concealment, due to heterogeneity in attack rates on leaf miners vs. gallers and borers; (2) Predation increases and disease decreases as succession proceeds (early vs. late) whereas parasitism is unaffected by successional status; (3) Predation and disease are greater in natural vs. cultivated habitats, whereas parasitism is unaffected by cultivation status; (4) Predation dominates in tropical/sub-tropical habitats, whereas parasitism does in the temperate zone. The herbivore's invasion status (native or non-native) is unrelated to mortality by any enemy type. Our results reiterate feeding biology as fundamental to herbivore population dynamics and identify several general patterns in insect demographics that should be useful for hypothesis testing.

10-039

INTRAGUILD PREDATION AMONG PREDATORS OF THE POTATO APHID; CHARACTERIZATION AND INFLUENCE OF THE EXTRA-GUILD PREY DENSITY.

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Intraguild predation (IGP), a common interaction among competing species of predators, influences both the abundance and distribution of members of a given guild. In the laboratory, within a system involving three species of aphid predators, *Aphidoletes aphidimyza* (DIP: Cécidomyiidae), *Chrysoperla rufilabris* (NEU: Chrysopidae) and *Coleomegilla maculata* (COL: Coccinellidae), we examined the role of predator size, mobility and diet breadth on the level and symmetry of IGP. Results indicated that (i) sessile stages were most susceptible to IGP, (ii) small individuals were more likely to be killed by large predators, and (iii) the aphid specialist *A. aphidimyza* suffered high risk of predation by generalist predators. We also examined the relationship between IGP and the abundance of an extraguild prey, the potato aphid *Macrosiphum euphorbiae*. Several patterns were observed along with increasing extraguild prey density. However, IGP sometimes remained elevated and constant at very high aphid densities.

10-040

COMPLEX POPULATION DYNAMICS IN A HOST-PATHOGEN-PARASITOID INTERACTION

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Whilst theoretical studies have encompassed multispecies interactions, empirical studies of these complex systems have been rare.

Here, a laboratory-based interaction between the Indian meal moth, *Plodia interpunctella* (Hubner) (Lepidoptera; Pyralidae), its granulosis virus, and the solitary endoparasitoid *Venturia canescens* (Gravenhorst) (Hymenoptera; Ichneumonidae), is described. Replicated populations consisting of the host alone, host-pathogen, host-parasitoid and host-pathogen-parasitoid combinations were established and monitored weekly to determine the patterns in abundance of each of the three species.

The host alone and the two-species systems predictably exhibited long term persistence and cyclic fluctuations of approximately one host generation in length. In striking contrast, the three-species populations were characterized by instability and almost inevitable extinction of the host and parasitoid. Furthermore, the increase from two to three species gave rise to a marked shift in cycle period in which both the host and parasitoid exhibited multigeneration cycles.

10-042

LOCAL DYNAMICS OF TROPHIC INTERACTIONS IN ACARINE SYSTEMS

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A lumped parameter model representing the dynamics of interacting species is presented; the model is described by a system of O.D.E. in terms of biomass with few observable parameters, with the aim of reducing the complexity of the system and of supporting practical use. Methods are proposed for estimating demographic and physiological parameters related to birth, growth and death processes as well as to food consumption and conversion. For this purpose numerical simulations with single-species age-structured models have been carried out.

The dynamics of the system is characterized by the growth function of the prey, the functional response of the predator, and death functions of both populations; these functions depend on both ecophysiological and behavioural parameters.

Basic model properties are studied with particular reference to functional response functions. The model is used to study the dynamics of acarine systems, relevant to poultry houses and protected crops.

10-041

FROM STABILITY TO CHAOS: CHANGES IN DYNAMICAL PROPERTIES IN 1 HOST-2 PARASITOID EXPERIMENTAL SYSTEMS

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Theories have predicted that "top-heavy" ecological systems with more predatory species than that of prey cannot persist for a long time. Assuming different functional responses between consumers, Armstrong and McGhee (1980) showed a long-term coexistence in an 1 resource-2 consumer system with non-equilibrium dynamics. The scenario had been presented by Utida's (1957) 1 host-2 parasitoid experimental systems.

Using the same organisms as Utida's, the present paper studied changes in dynamical properties of the 1 host-2 parasitoid systems based on time-series analysis and model simulations. Providing 10 g of azuki beans (*Vigna angularis*) at 10-day intervals, Shimada continued long-term population census. He started host populations first (a seed beetle, *Callosobruchus chinensis*), then introduced a pteromalid wasp, *Anisopteromalus calandrae* (A. c.) on day 150, and finally added a braconid wasp, *Heterospilus prosopidis* (H. p.) on day 440. The two wasps parasitize from the fourth instar larva to the pupa, though H. p. prefers a slightly younger host stages than A. c.. The attacking rate of A. c. depends strongly on host density per bean but not for H. p.. In larval competition between the two parasitoids, the first attacker is likely to be more advantageous irrespective of the species.

The population oscillated mildly in the host-A. c. system. After adding H. p., the host and A. c. increased gradually the amplitude of population fluctuations and, finally, the two populations fluctuated quite large like "chaos" in the 3 species system. H. p. population went extinct on day 1220 in a replicate system, then the host and A. c. returned original, mild oscillations soon.

We constructed two types of models. One is a matrix population model which includes competitions and parasitism, and the other is a delayed differential-equation model based on daily-based age structures. Using these, we examine which factor(s) and process(es) change the host and A. c. populations from stable to chaotic dynamics in the 3 species system with H. p..

10-043

EVOLUTION IN HOST POPULATION STRUCTURE PROMOTES PERSISTENCE OF HOST-PARASITOID SYSTEMS

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Many theoretical studies have suggested that population structures can promote persistence of otherwise non-persistent host-parasitoid systems. However, there have been very few empirical tests on their effects and none on their evolution. In this study, the effects of the spatial structure (local capacity, i.e., number of larvae per resource unit) and temporal structure (vulnerable period) in host populations on the persistence of host-parasitoid systems are quantitatively evaluated by laboratory experiments and by well-parameterized model analyses. Bruchid beetles, *Callosobruchus*, were used as hosts and *Heterospilus prosopidis* as parasitoid.

Local capacity: Fewer larvae are able to complete development in a bean in *C. maculatus* than in *C. chinensis*. Two long-term systems, *C. maculatus*-*H. prosopidis* and *C. chinensis*-*H. prosopidis*, were maintained, with constant resource supply for the hosts. The system with *C. maculatus* persisted longer, which is the 'paradox of enrichment' predicted by Rosenzweig.

Local capacity and vulnerable period: Two host species, *C. maculatus* (few larvae per bean) and *C. phaseoli* (many larvae per bean), and two kinds of beans, mung and azuki, were combined to construct four (2 x 2) host-parasitoid systems that differed in spatial and temporal structures. The persistence of host-parasitoid systems was promoted by the reduction either in local capacity or in vulnerable period, and the effect of local capacity, i.e., intraspecific competition, was greater. A model with host population structures supported this experimental result.

Evolution in host population structure: An increase in the system stability was observed around day 400. Host population structures were compared between before and after the long-term coexistence.

10-044

RANDOMIZATION TESTS FOR COMMUNITY ASSESSMENT

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Community parameters have seldom been evaluated with statistical tests because of discrepancy from statistical assumptions. However, randomization tests require less assumptions to test the community parameters. The randomization tests for community parameters are classified to two types; 1) a randomized community and 2) a randomization ANOVA and two pair tests..

The randomized community, in which individuals or species are randomly allocated to several communities or samples, was first adopted by Connor and Simberloff (1979) as a null-hypotheses to test an assembly rule of island birds. There have been controversy concerning the reality and logic of randomized community as a null-hypotheses. In the other test, the randomization ANOVA in which the parameters are randomly allocated to experimental blocks, observed Mean Squares are compared with randomized MS and it does not require the normality and homogeneous variance.

We tested these models for transect records of butterflies and quadrat samples of soil arthropods. Although it is suspected that the arthropods are distributed and found at random, our conclusion from the assumption of random community does not conflict with results obtained from other methods, such as a standard ANOVA and cluster analysis. The significant levels by the randomization ANOVA were higher than those by the standard ANOVA.

10-046

MONITORING OF Helicoverpa armigera (Hubner) WITH LIGHT AND PHEROMONE TRAPS

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Light and pheromone trap catch analysis for eight years from 1987 to 1994 and comparison with 1995 data indicated maximum activity of Helicoverpa armigera (Hb.) from October to December with a peak on 50th standard week at Raichur, India.

Trap catches preceded and succeeded by larval population and incidence on different crops. Egg population was coincided with same week and one week after the trap catches.

Maximum and minimum temperature and rainfall recorded highly significant negative correlation whereas morning relative humidity showed positive significant correlation with trap catches.

10-045

POPULATION MODELS WITH STOCHASTIC DISPERSAL, REPRODUCTION, AND MATE-FINDING: IMPLICATIONS FOR INSECT INTRODUCTIONS

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Deterministic, reaction-diffusion models suggest that Allee effects may be important barriers to biological control introductions. However, these models require one to assume that local heterogeneity in density, caused by stochastic dispersal, reproduction, and mate-finding, is unimportant. To test whether the conclusions of these models hold when this assumption is relaxed, a stochastic population model was developed that simulate development, survival, dispersal, mating, and reproduction of each individual in a population. The effects of mate detection distance, dispersal rate, and net reproductive rate on the numbers of insects needed to establish introduced species were compared for the two types of models. The potential for single-locus multiple-allele sex determination to prevent establishment or restrict spread of introduced parasitic Hymenoptera was also tested using a stochastic, individual-based model. The implications for biological control introductions are discussed.

10-047

CHANGES IN THE STAPHYLINID POPULATIONS IN THE CONIFEROUS FORESTS UNDER HUMAN IMPACT

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The successions of rove beetles (Coleoptera, Staphylinidae) in process of forest resumption in pine and cedar-silver fir forests of West Siberia were studied. The maximum diversity of rove beetles populations (56 species) were found in old cedar-silver fir forests. Tachinus rufipes, T.laticollis, T.marginellus, Philonthus decorus are predominate here. There are 35 species of rove beetles in the first stage of successions. Drusilla canaliculata and Philonthus lepidus are typical for open territory. Philonthus decorus, Xantholinus tricolor, some species of Tachinus and Atheta are predominate in young, 25-30-years forests. On the whole, staphylinid fauna at early stages of secondary successions are characterized by poorer variety of species and low quantitative

10-048

LONG-TERM DYNAMICS OF GRASSHOPPER COMMUNITIES IN THE FOREST-STEPPE LANDSCAPES OF WEST SIBERIA

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Long-term dynamics of Orthopteran (mainly grasshoppers) communities has been studied in natural and anthropogenic landscapes of the West-Siberian forest-steppes in 1981-1995.

Our data show that the general level of taxonomic diversity is more or less stable. On the contrary, grasshopper abundance fluctuates significantly and is evidently connected with climatic fluctuations. The maximum of grasshopper abundance on the forest-steppe, meadow and lawn plots have been observed during dry and hot summers of 1984-1985 and 1988. In the local agricultural landscapes, the dynamics pattern seems to be mainly determined by grasshopper migrations from more stable habitats.

10-049

EFFECTS OF INUNDATION ON BEETLE COMMUNITIES IN A DEGENERATED FEN GRASSLAND

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The structure and composition of the beetle community were studied in a degenerated fen grassland in Germany ('Großes Bruch', Saxony-Anhalt). Three different managed sites (intensively grazed pasture, extensively grazed pasture, hay meadow) were investigated by pitfall trapping during three years. A total of 343 beetle species with 39,400 individuals were collected.

From 1992-1994 a rapid decrease of numbers of species and individuals and a high species turnover were noted in all study sites. It is suggested that the seasonal inundation of 1993 and particularly in 1994 are responsible for the observed community dynamics.

By means of multivariate techniques it could be shown that the contrasts between the different managed sites of one year are smaller than the contrasts between the three sampling years in one site. This is a renewed reference to the problem of interpreting short-term data to characterize communities. Finally, the results are discussed in the view of ecological memory, inundation tolerance of species and in the diversity-stability relationship.

10-050

ANT COMMUNITY UNDER THE BEECH FOREST AT TANZAWA MOUNTAINS, CENTRAL JAPAN

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A study of ant community was done under the beech forest at Tanzawa Mountains, during June to September, 1995.

The research was made by using one-third diluted honey on aluminium dishes, which were set at the interval of 3-4 m and exposed during 90-120 minutes. Trapped ants were counted by species to make a table analysis.

The ant fauna under beech forest are classified in two types of community, one of which is *Myrmica kotokui* community and another is *Aphaenogaster japonica*-*Myrmica kotokui* community. They are corresponding to the associations of vegetation taxonomics, and segregated by the line of 1300 m of altitude. Namely, the former correspond to *Micicacalio*-*Fagetum crenata* (Miyawaki et al., 1964) and the latter correspond to *Corno*-*Fagetum crenata* (Miyawaki et al., 1964). These ant communities are classified again in two subtypes by moisture condition, that is, wet and semi-arid. Ant subcommunities under semi-arid beech forest which look like marginal with shrubby components, include usually *Lasius japonica*.

10-051

REACTION OF RIPICOLIC CARABIDAE AND ANTHICIDAE (COLEOPTERA) OF THE RIVER RHINE TO IRREGULAR FLOODING, AND THEIR SPATIAL AND TEMPORAL ECOLOGICAL NICHES.

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The banks of the river Rhine near Gernsheim, (Hesse, Germany) are broad, with well developed zones of gravel, sand and mostly natural vegetation. Due to the variation of the water level of the river, the investigation of the population dynamics of the riparian arthropods has to be carried out with an unusually high spatial and temporal resolution. Pitfall traps were set in transects perpendicular to the water's edge with an average separation of approximately 4 m. The traps had to be changed at an interval of 2-4 days (depending on variation of the water-level). The trap locations were not only described by their static environmental properties (e.g. substratum and vegetation), but also dynamic values (e.g. humidity, distance to waterline). Thus it was possible to correlate shifting species populations with environmental changes. With the help of multivariate statistical methods (principal component analysis; canonical correspondence analysis) ordinations of the traps were carried out to find ecological guilds. In contrast to the literature it could be shown that the river bank fauna is autochthonous. Two main ecological types could be defined according to their migration behaviour:

- 1) statically zoned species: These species are bound to characteristic static (as a rule in higher laying) Zones of the riverbank. Migration is not dependant on the water level.
- 2) dynamically zoned species: These species migrate to maintain a more or less constant distance to the waterline. This behavior is switched off only in winter.

10-052

INSECTS AS BIOINDICATORS IN
NATURE CONSERVATION AND LANDSCAPE PLANNING

- COMMENTS ON TAXA-SELECTION, SAMPLING METHODS AND INTERPRETATION

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Bioindication is a rapid-growing field of activity in applied entomology. In Central Europe, the use of insects as passive monitor-organisms in evaluation of nature conservation measures and in environmental assessments (EIAs) is already today's standard. Though the principles of selecting the used insect-taxa as well as the choosed sampling and interpreting methods are often insufficient or inadequate.

Each insect taxon is in principle qualified for being used as bio-indicator, unless insuperable taxonomical or methodological problems are existing. Obviously the sampling methods must be corresponding to the selected taxa and to the statement of the question. Today's greatest problem of entomological reports is the preperation of results: reproducible interpretations founded upon previously fixed, as far as possible universally valid (i.e. for other areas as well as for comparable taxa) criterions are not standard yet.

Development of quality control (minimum standards, criterions concerning comparability) in entomological reports - which will become an important field of activity for future entomologist as well as a valuable data ressource - is a very important scope of applied entomology in the beginning third millenium.

10-054

DYNAMICS OF A WEEVIL (COLEOPTERA:
CURCULIONIDAE) POPULATION ON A TROPICAL RAIN
FOREST

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The Phyllotrox spp. complex is the most abundant with 4764 specimens, while Camarotus sp2, with 4 specimens only, is the least collected species. During our 4 years survey the increment of the population occurred usually during the rainy season, most specially during the monthly of April, May, and June. This was true for all 10 species, thus showing that insects present seasonality in the Tropical rain forest. Seasonality and abundance seem to be mostly correlated with rain fall, while temperature, relative humidity, and wind speed seem to play a much lower role in those parameters. Interesting enough weevil yearly relative abundance correlate well with rainfall. In Barro Colorado Island, with very well delimited rainy and dry seasons, weevil abundance shows a maximum during the first rainy months, decreasing there after. Insect abundance also correlates with the altitude where the traps were placed, being higher in the 27 meters trap.

10-053

HOW MANY CARABIDS? GENERAL PATTERNS OF DENSITIES.

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The ground beetle literature is replete with 'density' data collected by methods which are likly to give inaccurate results. We have conducted a critical, world-wide review of carabid densities, including only data obtained by soil sampling, soil flooding, mark-recapture, fenced pitfalls, quadrat sampling and vacuum sampling. Such data are more numerous than generally acknowledged by carabidologists. Our database contains >250 records of 71 adult and 13 larval taxa, collected in 14 countries in Europe and North America. Small species (<5 mm) have density ranges of 0.2 - 77 individuals per sq. m, large species (>5 mm) range between 0.02 - 33 ind. per sq. m. Suitable hibernating sites regularly held very high densities (500-1113 ind. per sq.m). The total ground beetle densities average 31.7 ind. per sq.m in arable habitats, and 233.3 ind. per sq.m in field boundaries. Fewer data are available on larvae; total larval density ranges between 29.4 - 87 ind. per sq.m, that of individual species between 0.07 - 42 ind. per sq. m. Trends and patterns according to season, geographic location, habitat type, food habits, and species size are described and discussed.

10-055

INFLUENCE OF REWETTING AND INUNDATION OF FEN
GRASSLAND ON SELECTED GROUND BEETLES (COLEOPTERA:
CARABIDAE)

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During the last decades the intensified use of fen grassland (mineral and organic fertilizing, draining etc.) caused fundamental changes in the vegetation, accompanied by the loss of original ground beetle communities. The hygrophilous carabids of the wetlands disappeared and were replaced by eurytopic species, frequently with a high density of individuals.

In a three-year-field experiment in the Dümmer lowland (Northwest Germany, Lower Saxony) with four enclosures (each 500m²), the influence of rewetting and seasonal inundation was investigated on *Pterostichus melanarius*, *Poecilus versicolor*, the most abundant carabids in the research area, as well as *Carabus granulatus*. Two enclosures were moistened by ditches, two non-moistened used as control, the cutting treatment took place twice a year. The complete range was flooded during the winter. Grinding-marks on the elytra made it possible to distinguish the beetles individually. According to the rate of captured and recaptured beetles, body mass, amount of young individuals and phenology, different population parameters could be estimated. The ecological consequences of a modied water management on carabid populations in degraded fen grassland will be of interest in course of changing agricultural land use by changing areas from intensive cultivation to extensive use of grassland.

This research is supported by the German Ministry of Education, Science, Research and Technology

10-056

NON-TARGET EFFECTS OF A BROAD-SPECTRUM VETERINARY AGENT ON DUNG INSECT COMMUNITIES

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The increasingly widespread use of broad-spectrum veterinary agents has been considered to pose a serious threat to the dung-degrading insect fauna and consequently to the ecology of pasture ecosystems. This is the first study of its kind to investigate the ecotoxicological effects of the parasiticide ivermectin at dung insect community level.

Two field trials were conducted to examine short- and longer-term effects of ivermectin on dung insect communities under normal extensive farming conditions in South Africa. Different community measures (univariate, graphical and multivariate) were used to assess the impact of the drug. Under drought conditions ivermectin appears to affect dung insect communities for up to three months after treatment, primarily through a reduction in Shannon's species diversity and an increase in species dominance compared with untreated controls. No effect was found one year after treatment. Under more favourable weather conditions with relatively high rainfall, little or no effect was observed.

The results of the field studies suggest that the seriousness of the impact of ivermectin on the dung insect fauna depends on several factors, including climatic conditions, spatial scale of treatment (i.e. size of paddocks) and number of animals treated in a herd.

Laboratory assays indicate that ivermectin could have adverse effects on populations of the dung beetle *Euoniticellus intermedius*. However, the field trials have shown that populations of *E. intermedius*, the most abundant scarabaeine species at the study site, appeared unaffected under both drought and high rainfall conditions.

10-057

DYNAMICS OF GRASSHOPPER (ORTHOPTERA: ACRIDIDAE) COMMUNITY IN RESPONSE TO LIVESTOCK GRAZING ON ARID STEPPES OF INNER MONGOLIA

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Plant and grasshopper community variables were monitored on the natural steppes grazed by livestock in Inner Mongolia. Biomass, richness and abundance of the plant and grasshoppers were compared between different seasons and between grassland sites under different grazing intensities. Change in plant community structures directly affected grasshopper species composition and abundance. Grasshopper abundance and diversity were negatively correlated with plant biomass and diversity in early growing season, positively in middle season, and no correlation in late season. Moderate grazing could preserve more diverse grasshoppers with lower proportion of pest species. The vegetation played a more important role as grasshopper habitat than as food resources. Soil compactness and water content also significantly affected grasshopper abundance and richness. Multidimensional resource utilization in grasshopper assemblages was analyzed. There were sufficient differences between grasshopper species for overall overlaps associated with resource use to explain coexistence in the assemblages by resource segregation. Grasshopper species-specific use of resource may be due to primarily adaptive differentiation and to coevolution interactions between grasshopper and plant rather than interspecific competition among grasshopper species. The significance of some grasshopper indicators to the change of grassland environment was discussed.

10-058

LOCUST SITUATION IN THE ZONE OF THE ARAL SEA ECOLOGICAL CATASTROPHE

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The delta of the Amudarya river, situated to the south-east of the Aral sea, was one of the most important permanent breeding zone of the Asiatic Migratory locust, *Locusta migratoria migratoria* (L.), in the former Soviet Union. The area of reeds that could be colonized by this locust was as high as 14 million km². In the outbreak years, it was necessary to control the Migratory locust on 500,000 ha. Another species of economical importance, the Italian locust *Calliptamus italicus* (L.), was restricted only to the irrigated zone. It colonized the habitats of the anthropogenous origin (road-sides, fallows, uncultivated lands with weeds etc.). The drying of the delta after the excessive uptake of water from the Amudarya river for irrigation which began in the 1960s, has led to the radical changes in the environment, and, consequently, in the locust situation. The delta was subject to rapid desertification. The area of the humid habitats (reeds) dramatically decreased. The fluvial forests are at the limit of extinction. The mesic Orthoptera species were substituted by the xeric ones, and their diversity declined: out of twelve species registered from this zone in the sixties, only seven were found in 1990-1993. The habitats favourable for *L. m. migratoria* are reduced, its economical importance became of the second level. On the contrary, the environment became most favourable for *C. italicus* which, due to its ecological tolerance and broad polyphagy, colonized readily the vast surfaces menacing the crops of value (cotton, rice). To protect them, it is necessary to apply chemical treatments over the areas of hundreds of thousand ha annually. This aggravates the risk of environmental pollution in the conditions of ecological catastrophe. Possible alternatives to the curative chemical locust control in the region, including the use of bio-pesticides and the methods of cultural control, are discussed.

10-059

INTRA- AND INTERSPECIFIC AGGREGATION OF DUNG BEETLES (COLEOPTERA: SCARABAEOIDEA) IN ALPINE PASTURES

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Spatial distribution of scarabaeoid beetles on dung pats was studied in the Argentera Natural Park (south-western Italian Alps) from June to October in 1994 and 1995. Artificial dung pats were distributed evenly within the study area (about 1400 m. a.s.l.) and sampled at suitable dung ages (1-13 days). Two experimental tests were also carried out. In the first test experimental pats were covered with metallic nests which prevented the largest species from arriving at the dung, whereas in the second one a variable number of individuals of a certain species was added to experimental pats after their distribution in the study area.

The community was dominated by *Aphodius* species (20 out of 25 species), which usually were also the most abundant in the pats. Geotrupidae were represented by only two species and Scarabaeidae by three. The period of colonization of the dung was very short since pats older than seven days housed virtually no individuals.

Patterns of spatial distribution showed some degree of intra- and interspecific aggregation. Preliminary results suggest that resource quality, resource detectability, and soil type did not significantly affect interspecific association. In fact species seemed to have an intrinsic disposition for aggregation, maybe dependent upon not specific signals from any abundant species aggregating in a dropping. Cost and benefits of such a behaviour are briefly discussed.

10-060

PRIMARY SUCCESSION AND COMPOSITION OF CARABID POPULATIONS ON A COAL MINING WASTE DISPOSAL (COLEOPTERA: CARABIDAE)

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In Central Germany the landscape of highly intensive agriculture is interrupted by many waste hillsides and mines from mostly finished coal mining. On one of these hillsides investigations over a period of four years were carried out to follow the changes of carabid populations in different habitats by means of pitfall traps. Eventhough in none of the habitats a constant increase in species and individuum numbers over the whole period was evident, the trapped beetles indicated an ongoing succession. Where vegetation, inclination, soil acidity etc. were closer to the conditions on the surrounding fields many species in high numbers appeared. In more extreme habitats a slower and different kind of succession was found.

10-062

THE TEMPORAL DYNAMICS AND THE ADULT MOVEMENT OF CLAVIGRALLA TOMENTOSICOLLIS STÅL (HETEROPTERA: COREIDAE) IN BENIN'S COWPEA FIELDS WITH REFERENCE TO TRAP CROP EFFECTS ON IMMIGRATION

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The temporal dynamics and the adult movement of *Clavigralla tomentosicollis* Stål were related to cowpea phenology using visual counting and mark-recapture methods. Colonization by immigrating adults closely coincided with cowpea pod formation and ceased thereafter. Analyses of adult movement yielded daily emigration rates of 7 to 20% of the resident adult densities. Daily in-situ mortality of adults was below 5%. The decline in adult numbers towards harvest is thus mainly due to emigration. Given the high mobility of *C. tomentosicollis* the effect of pigeonpea trap crops on adult movement was tested. Immigration rates of *C. tomentosicollis* adults were generally smaller into cowpea fields with traps than into cowpea fields without traps.

10-061

THE ECOLOGY AND POPULATION DYNAMICS OF AN ENDANGERED WEEVIL (*HADRAMPHUS SPINIPENNIS*) ON THE CHATHAM ISLANDS, NEW ZEALAND - A CONSERVATION APPROACH
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H. spinipennis belongs to a small genus of large, flightless weevils (Family Curculionidae). Its distribution is restricted to two predator-free outlying islands of the Chatham Islands group (New Zealand), but it is believed that the weevil had a much wider distribution before rodents and cats were introduced. The phenology, ecology and population dynamics of *H. spinipennis* have been investigated in the summers of 93/94 and 95/96, during five visits to one island. The adult weevils reach at least two and a half years of age and feed on foliage of *Aciphylla dieffenbachii*, Apiaceae. The larvae develop on the roots. The impact of the feeding weevils and larvae has caused the disappearance of *Aciphylla* patches in the past, leading to a fragmental, but interacting metapopulation of weevils. One subpopulation was chosen for an intensive mark-recapture study. The plants in one patch were mapped annually and a total of over 9000 weevils were marked individually. The number of weevils in the patch increased rapidly from just over 2000 individuals in the summer of 93/94 to over 6000 in the summer of 95/96 causing a decline in the number of host plants from 625 adult plants to 250. For the adult weevil population, death and survival rates were calculated as well as the dispersal rates within the patch. The future demographic development of the studied weevil subpopulation, its interaction with the population dynamics of the host plant and its dispersal ability are discussed. By using the results of this study a management plan for this endangered weevil species has been developed.

10-063

ESTIMATES OF POPULATION SIZE AND DISPERSAL RATES OF THE SHEEP BLOWFLY *LUCILIA SERICATA* FROM MARK RELEASE RECAPTURE EXPERIMENTS.

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Mark release recapture studies were carried out using the ectoparasitic sheep blowfly, *Lucilia sericata*, in sheep pastures in South West England. Marked flies were released and recaptured using liver baited sticky targets in concentric rings surrounding a central release point. Estimates of the maximum and average distance travelled were obtained by fitting a model to the number of flies recaptured against distance from the release point. The results show that flies dispersed in an approximately random pattern from the release point at an average rate of approximately 100m per day. Estimates of the size of the wild population were derived using the Lincoln index, after correcting for underlying rates of mortality and emigration of marked flies. The results show that *L. sericata* populations increase in abundance throughout the summer but, even peak population densities are relatively low. The implications of these results for sheep blowfly control will be discussed.

10-064

COMPETING RISKS ANALYSIS, RISK ASSESSMENT, AND SIMULATION OF POTATO TUBER MOTH *PHTHORIMAEA OPEECULELLA* (ZELLER) (LEPIDOPTERA:GELECHIIDAE) MULTI-COHORT SYSTEMSO. Roux & J. Baumgärtner¹

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The larvae of Potato Tuber Moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) mine potato foliage and borrow deep galleries into tubers. In Tunisia, PTM is a major pest in rustic shelter of potato tubers.

The separation and integration of mortality factors is a prerequisite for the design of a pest management system. For a single cohort system, elements of the competing risk theory were incorporated into the analysis of cohort survival. Thereby, each mortality factor is characterized by a force of mortality and a corresponding survival distribution function. Mortality factors are combined using forces of mortality in order to describe different states of the PTM single cohort system.

Subsequently, the variability of the effect of the most important mortality factor is introduced into the system and the resulting risk for damage is analyzed.

To illustrate the effect of varying mortality factors on the dynamics of multi-cohort population systems, we use a simulation model based on time varying distributed delays.

10-066

DISTRIBUTED DEVELOPMENTAL TIMES OF POIKILOTHERM COHORT INDIVIDUALS AND THE USE OF THE ERLANG FREQUENCY DISTRIBUTION

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Under constant temperature the observed exit flow from an arthropod life stage, illustrated by immature development of the boll weevil *Anthonomus grandis* Boheman (Coleoptera, Curculionidae), can adequately be described by a recursively expanded stage balance equation. The resulting equation has the ERLANG frequency distribution as solution, that depends on two parameters: the first parameter represents the number of substages within a life stage, while the second parameter is the probability for an individual to move from one life stage into the succeeding one.

Published data on several arthropod cohorts are used to test the validity of the ERLANG frequency distribution for representing the observed distribution of developmental times.

10-065

ANALYSIS AND MAPPING OF SPATIAL DISTRIBUTION OF MASSON-PINE CATERPILLAR, *DENDROLIMUS PUNCTATUS* (WALKER), (LEPIDOPTERA: LASIOCAMPIDAE), IN DIFFERENT PINE FORESTS USING GEOSTATISTICS

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Studies were conducted in 1995 to investigate the spatial distribution of the pupae and larvae densities of *Dendrolimus punctatus* (Walker), as well as the parasitic guilds of pupae in three different types of pine forest (i.e., pure masson pine stands of high and low caterpillar population density, and mixed pine forest). Samples were collected in 20x20 and 36x12 grid in these pine forests. The regionalized variables and kriging technique in geostatistics were used to assess the spatial pattern of *D. punctatus* and its parasitic guild. Geostatistical analysis offers advantages over traditional indices of spatial dispersion. Kriging technique makes optimal, unbiased estimates of regionalized variables at unsampled locations using initial set of data values and the structural properties of the semivariogram.

These analyses revealed spatial distributions of pupae, larvae and parasitic guilds of pupae in three pine forests, which varied as pine caterpillar density and different pine forests. In mixed pine forest, semivariograms of pupae and larvae were fitted by spherical model ($r^2=0.923$ and $r^2=0.879$, respectively), indicating an aggregated spatial arrangement. Their localized discontinuity was 0.354 and 0.277 at 5m scale, respectively. Spatial dependence occurred at a range of 38.7m and 13.9m, respectively. Semivariogram of pupae parasitic guild was fitted by level linear model, sill was 0.297. In pure masson pine stands of high density, semivariogram of pupae was fitted by linear model with no slope, localized discontinuity equals to sill, which is 1.42; in low density stands, semivariogram was linear with a very low slope 0.072, indicated random or uniform spatial pattern. The punctual kriging procedure can use these semivariograms to generate maps of interpolated estimates of pupae and larvae densities in the mixed forest. The results and methods will be useful for insect pest management of forest in developing sample maps and understanding the spatial relatedness of pine caterpillar populations.

10-067

METAPOPOPULATION STRUCTURE OF *CARABUS PROBLEMATICUS* HBST. (COLEOPTERA, CARABIDAE) IN A FRAGMENTED LANDSCAPE, - SIMULATION RESULTS IN COMPARISON WITH FIELD DATA.

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The ground beetle *Carabus problematicus* is a species usually living in forests, but exhibiting a certain degree of dispersal activity outside wooded habitats, too. Within an intensely cultivated agricultural landscape in Germany the spatial distribution patterns of this species were studied for three years (1992, 1994 and 1995). In this region, today only a few, mostly isolated patches of woodland have remained. *Carabus problematicus* was only caught in large forest remnants or in forest islands not too much distant from these. However, in some of the smaller and more remote forest patches the species could not be found every year. This finding (result) is interpreted as an indication for the existence of metapopulation dynamics of *C. problematicus* in this landscape area.

On the basis of a GIS generated map containing the spatial distribution of suitable habitats at landscape scale, of own field results on the occurrence of this species and of informations from literature on different biological population parameters, a metapopulation model is developed using the program RAMAS-GIS. Simulation results obtained are corresponding well with the field data. According to the model the spatial arrangement of habitats and the distance between neighbouring occupied habitat patches belong to the most important parameters stabilizing populations of *C. problematicus* at landscape level. Applicability and limitations of the model as well as consequences for nature conservation planning in agricultural landscapes are discussed.

10-068

INVESTIGATION OF THE FEEDING PREFERENCES OF *STETHOPHYMA GROSSUM* (ORTHOPTERA: ACRIDIDAE) IN FIELD AND LABORATORY EXPERIMENTS

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In field and laboratory experiments both sexes of *Stethophyma grossum* were tested for differences in their feeding preferences because of the differences in their body size and behavior.
Therefore diverse species of grass were offered under standardized conditions. As expected, females need more food on a account of their larger body size.
But the study also shows that males and females of this species prefer the same grasses. As *St. grossum* is limited to wetland it is interesting that in no offered combination a grass of the species *Carex* was preferred.
For the field experiments the test animals had to hunger for at least two days. These grasshoppers seem not to be as selective as in the laboratory. So in some cases even straw and herbs were tested for a short time until a grass was chosen twice.

This research was supported by the German Ministry of Education, Science, Research and Technology under Grant BEO-0339559

10-070

NEW BIOLOGICAL DATA ON THE APTEROUS BEETLE *ELYTROSphaera LAHTIVIRTAI* BÉCHYNE (CHRYSOMELIDAE: CHRYSOMELINAE) IN BRAZIL.

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The biology and ecology of the apterous genus *Elytrosphaera* is poorly known. It was only recently that P. Jolivet and J. Vasconcellos-Neto published two papers, one dealing with the biology of *E. xanthopyga* and another with the genus distribution. The subgenus *Elytrosphaera* s. str. occurs mainly on the Brazilian plateau (8 species) and one occurs in Bolivia.
Elytrosphaera lahtivirtai is an endemic species at Itatiaia National Park, Rio de Janeiro state, Brazil. We found both larvae and adult of this species feeding on *Solanum caeruleum* Vell., the first record of this genus on Solanaceae. *Adenostema brasilianum* (Asteraceae) was previously recorded as the host plant of *E. xanthopyga* at Viçosa, Minas Gerais state, Brazil.
E. lahtivirtai occurs at near 1000m altitude where it is apparently more common on plants near streams. Adults appear in the field during November, reproducing from December until February, and disappearing in March. The adults probably diapause in the soil until the following spring.

10-069

HOST PLANT PREFERENCE IN *PLAGIOMETRIONA* SP (COLEOPTERA: CHRYSOMELIDAE: CASSIDINAE)

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At Poço das Antas Biological Reserve, Rio de Janeiro State, Brazil, *Plagiometriona* sp has been observed feeding, throughout the year, as both larval and adult forms, on two Solanaceae species: *Aureliana fasciculata* (Vell.) Sendt. and *Acnistus arborescens* (L.) Schecht. In order to evaluate host plant preference, two sites were chosen where, in one of them *A. fasciculata* was about twice more abundant than *A. arborescens*, in the other site *A. arborescens* was about four times more abundant than *A. fasciculata*. Seven individuals of each plant species were marked in each area and numbers of immature and adult beetles were recorded in three different seasons: winter (July, 1995), spring (October, 1995) and summer (January, 1996). In general, *A. fasciculata* was significantly preferred by the beetle larvae regardless of the plant relative abundance. Adults as a whole did not show any significant difference in numbers on the two host plants, except for the spring where they occurred more often in *A. fasciculata* in the area where *A. arborescens* was more abundant. This preference may be due, in part, to the higher abundance and the wider distribution of *A. fasciculata* in the reserve. Other possible explanations, related to the host plant quality, are being investigated. Financial Support: CNPq and Fundação O Boticário de Proteção à Natureza.

10-071

A SPECIAL CASE OF CAMOUFLAGE IN A TROPICAL CURCULIONID BEETLE, *PERIDINETUS ZINCKENI*

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Adults of *Peridinetus zinckeni* Rosenschold, 1837, are often found in Southeast Brazil, feeding on Piperaceae leaves. When feeding, during the night, this beetle leaves several circular holes, already recorded for *P. sellatus* elsewhere.
P. zinckeni also produces elliptical holes inside which it rests laterally during the day. The elliptical holes occur in a much lower density than the round ones. The rest is made in such a way that half of its black and white body can be seen in each side of the leaf. Besides this, the insect also defecate in each extremity of the elliptical resting hole producing an image consisting of three black dots quite similar to bat feces, frequently observed on the leaves of *Piper*. It is likely that this behavior of *P. zinckeni*, already observed in *Piper arboreum*, *P. gaudichaudianum*, *P. amplum* and *P. mollicornum*, corresponds to an adaptive camouflage. Even though such a curious behavior has not yet been recorded, it may occur in other insects with similar biological aspects and host plants, such as other Baridinae species. Financial support: CNPq, FUJB/UFRJ, Fundação O Boticário de Proteção à Natureza.

10-072

HISTORICAL ETHOLOGY IN THE EMPIDOIDEA (DIPTERA: HETERODACTYLA: EREMONEURA)

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The phylogeny of the Empidoidea is briefly discussed in comparison with the works of different authors, especially Chvála (1981, 1983), Cumming et al. (1995) and Sinclair (1995). The questions of the monophyly of the Empididae and the validity of the Hybotidae are especially approached.

Several ethological characters including the predaceous or nectar-feeding habit, the presence or absence of swarms, the mating activity on the ground or in the air, the presence or absence of the 'nuptial gift', the nature of the gift... are analyzed in the Empidoidea in reference to their phylogeny. The method consists to map the ethological characters or attributes (traits upon which *a priori* homology and polarization are so hazardous that one prefers to make these operations in reference to an extant phylogeny, Grandcolas et al., 1994) on the cladogram to follow their evolution. It is an objective test of the classical hypotheses relating the reproductive behavior as regards the traditional scenarios (Poulton, 1913, Kessel, 1955) and the sexual selection.

It is more particularly considered that the 'nuptial gift' has not appeared repeatedly in the Empidinae but that this behaviour has an monophyletic origin. The phylogeny hypothesis of the subfamily provides evidence that the subgenera *Xanthempis*, *Lundstromiella* and *Holoclera* are groups exhibiting numerous derived characters contrarily to the classical hypothesis, consequently the absence of the 'nuptial gift' in these subgenera is considered as the result of reversals.

All other attributes are analyzed according to the same method, in reference to the phylogeny and as regards the parsimony principle.

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10-074

THE ASSEMBLY OF A THREE-LEVEL FOOD CHAIN IN WATER-FILLED CONTAINERS WITHIN FOUR AUSTRALIAN, SUBTROPICAL RAINFORESTS

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A number of water-filled containers were placed in subtropical rainforest along a short latitudinal gradient in four regions of Eastern Australia. Several dipteran species selected the containers as oviposition sites. Among these species, the predaceous larvae of *Anatopynia pennipes* (Freeman), a tanypodine chironomid, feed predominantly on larvae of the mosquito *Aedes notoscriptus* (Skuse). The mosquito colonized habitat units within the first three months in all four regions while the tanypodine chironomid was generally slower to colonize. Initial colonization by *A. pennipes* took longer at higher latitude. Consequently, a three-level food chain took twice as long to form in container habitats in the forests at higher latitudes. I propose that seasonal droughts act as a disturbance to depress populations of *A. pennipes* in the higher latitude forests.

10-073

ZOOSAPROPHAGY AND PHYTOSAPROPHAGY IN MYRMECOPHILOUS CHRYSOMELID BEETLE LARVAE, *MACROLENES DENTIPES* AND *PACHYBRACHIS ANOGUTTATUS* (COLEOPTERA: CHRYSOMELIDAE: CRYPTOCEPHALINAE)

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Controversy exists about the nutritional ecology of larvae of the chrysomelid tribe Clytrini. Previous studies resulted in two opinions, claiming that these myrmecophilous larvae are either phytosaprophagous and/ or predators of ant progeny. Little is known about larvae of the tribe Cryptocephalini living in the vicinity of ants.

Food selection of the larvae of two species found in the vicinity of an ant nest in Mallorca, Spain, *Macrolenes dentipes* OLIVIER (Clytrini) and *Pachybrachis anoguttatus* SUFFRIAN (Cryptocephalini) was studied in the laboratory.

In multiple choice tests, dried and rehydrated leaves of *Quercus ilex* and dead larvae and adults of the Lepidoptera, *Ephestia kuehniella*, were offered.

Whereas the larvae of *P. anoguttatus* fed exclusively on wet and dry leaves of *Q. ilex*, the larvae of *M. dentipes* fed on all four substrates offered and showed no significant preference for vegetable or animal material.

The data support the hypothesis that in the Clytrini, the larvae feed on remnants of prey deposited by the ants in certain areas of the nest.

10-075

THE SIGNIFICANCE OF DITCHES FOR *STETHOPHYMA GROSSUM* (ORTHOPTERA: ACRIDIDAE) WITH SPECIAL CONSIDERATION OF DISPERSAL

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A mark-release-recapture experiment was carried out in order to see if the dispersal of *Stethophyma grossum* is influenced by the presence of a drainage ditch (within a relatively unsuitable habitat). Additionally, by walking on 100m transects, about 150 ditches together with the adjacent areas were mapped with regard to colonization by *S. grossum*. Further parameters describing the structure and vegetation of the ditches were also recorded.

As for the dispersal of *S. grossum* when released at a single point, it seemed to be insignificant whether the releasing-point was situated directly at the borders of the ditch or at some distance from it on the meadow. Furthermore, consistently fewer individuals were found within a distance of 1m from the water's edge than were on the adjacent land. Correlations between „ditch type“ and the numbers of individuals occurring on the ditch borders could not be ascertained as they were either non-significant or extremely weak. However, a strong correlation was found between the numbers of *S. grossum* on the ditch borders and on the adjacent areas.

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10-076

THE DEVELOPEMENT OF A HABITAT-SUITABILITY-MODEL FOR *CONOCEPHALUS DORSALIS* (ORTHOPTERA: TETTIGONIIDAE)

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For the scientific assessment of areas it is increasingly important to be able to make quantitative statements using objective measurable parameters.

In this study the special requirements of *C. dorsalis* regarding habitat quality serve as the main parameters.

Using geographic information systems (GIS) certain structural types were ascribed to the plots of the research area in a fen.

921 test sites and 289 trench borders were selected and investigated with regard to significant key factors (e.g. plant species, plant height, land-use, etc.) and abundance of these regional target species.

The frequency and density of *C. dorsalis* were determined for a wide range of parameters in each case. The results were applied to develop a habitat-suitability-model.

The relationships described by this model should supply the basis for a simple or quick forecast for the habitat-quality for *C. dorsalis* in an area which has not been investigated up to the present.

This research was supported by the German Ministry of Education, Science, Research and Technology under Grant BEO-0339559.

10-078

ON THE CLASSIFICATION AND EVOLUTION OF ECOLOGICAL NICHES OF INSECTS

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Ecological niche is the comparatively homogeneous functioning sphere of living organisms in physical, chemical and biological respects that bring about the necessity of formation of special adaptations for the existence and development, first of all for feeding and movement. At present the classification of ecological niches is absent. They can be divided into two types: aquatic and terrestrial niches. Soil surface, soil and litter, surface of the seed plant above-ground organs, plant tissues, body surface, tissues and internal organs of animals are the main subtypes of insect terrestrial ecological niches. Formation of the highest taxa of insect life-forms is related with exploitation by them of main and complementary (caves, burrows, nests and excrements of vertebrates, nests of ants and termites, fungi carposomes etc.) ecological niches. Insects came off the aquatic ancestors on the soil surface with loose plant remains near the water bodies. Then they settled plant tissues, soil, animal body surface and tissues, burrows and nests of vertebrates. Insect transition to development from one type and subtype ecological niches another had taken place more than once.

10-077

ECOLOGY OF A GALL WITH GLANDULAR TRICHOMES IN *CUPHEA CF CALOPHYLLA* (LYTHRACEAE), IN BRAZIL

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The family Lythraceae has only two types of entomogenous galls described for the Neotropical region, both of them occurring in plants of the genus *Cuphea*. This study has been carried out at Poço das Antas Biological Reserve, Rio de Janeiro state, Southeast Brazil. There, we find *Neolasioptera* sp (Diptera, Cecidomyiidae) galling apical shoots and stems of *Cuphea cf. calophylla*. The gall has several chambers containing one larva per cell. It is densely covered by glandular trichomes, absent at the beginning of the gall development, which show intense glandular activity when the gall is fully developed. The purposes of this work are to describe the biology of the gall maker, the fluctuation of its population, and the possible role of the glandular trichomes on the protection for the gall maker against its natural enemies.

We have chosen sites to obtain gall makers and parasitoids, where twice a month we have been collecting galls. The latter occur throughout the year, showing an increase of its abundance during the summer. Experiments in field and laboratory are being carried out to test the possible role of the trichomes in the protection of the gall maker.

Up to now we have obtained only one parasitoid species, Eulophidae, which emerges during the late development of the larva or in pupae. Parasitism rate was relatively low for all the period of study. Many chalcid wasp species have been found attached to gall trichomes. This observation as well as the relatively low parasitism rate suggest that trichomes may have an important role on the gall maker survivorship.

10-079

COLEOPTERA: ELATERIDAE, CANTHARIDAE AND MALACHIDAE AREAS WITH STOPM DAMAGE IN SWITZERLAND

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Beetles of the Elateridae, Cantharidae and Malachidae were sampled in two different regions (Dissentis, Limpachtal) in four habitats and four different traps. The traps were controlled once a week between the period from 01.05. to 01.10. during the years of 1987 to 1990. Statistical analysis produced interesting results of the distribution of the insects in time and place as well showed as meaning of areas with storm damage for the fauna of specific beetles.

10-080

NUTRITIONAL PREFERENCE OF THE DUNG BEETLE, *SCARABAEUS CRISTATUS* F. (COLEOPTERA - SCARABAEIDAE) FROM KUWAIT

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Adult *Scarabaeus cristatus* F. consumed the fluid components of the dung; when three herbivorous animal dung such as horse, camel and sheep were offered, the beetles preferred the horse dung to the others. The sheep dung was preferred to the camel dung. The carnivorous animal dung, such as that of dog and fox were also consumed but to a lesser extent. The dog dung was preferred to the fox dung. As a whole, the herbivorous animal dung was preferred to the carnivorous one. The physical and the chemical characters of the dung play an important role in the dung consumption by the beetle. The odour of the dung is a vital clue that guides the dung beetles to its food.

10-082

PRELIMINARY REPORT ON HIGH TEMPERATURE TOLERANCE OF RICE LEAFFOLDER, *CNAPHALOCROCIS MEDINALIS*R. Zhang¹, K.L. Heong, I.T. Domingo²

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The high temperature tolerance of rice leaffolder (RLF), *Cnaphalocrocis medinalis*, was measured in temperature range between 34 and 40°C, 70%RH, and 12:12 illumination and the quantal response data were subjected to probit analysis using the computer program developed by Finney. The median lethal dosage (LT₅₀, expressed in hours) was used to determine the survival ability of RLF in high temperature conditions.

Different values of LT₅₀ were found among stages of egg, larva, pupa, and adult. The highest LT₅₀ is in larva stage (186.5 hours) whereas the lowest exists in the stage of egg (30.1 hours). At range of 35 and 40°C, LT₅₀ of eggs decreased from 69.9 to 30.1 hours with increasing temperature, which was described by the linear regression equation in form of $LT_{50} = 330.9 - 7.6T$. The experimental results also showed that there are no significant differences in LT₅₀ among three different local populations.

10-081

DUNG BEETLES ASSEMBLAGES INHABITING BOVINE AND OVINE DROPPINGS IN URUGUAYAN PRAIRIES

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Dung beetles have been studied along a two year period (January 92-April 94). Monthly samples were taken by directly examining bovine and ovine feces (480 bovine feces and 330 ovine feces). A grand total of 1846 individuals in 12 different species has been obtained (1279 individuals in 12 different species in bovine droppings and 567 individuals and 11 species in ovine droppings). The most abundant species are *Onthophagus hirculus* (488 ex. and 115 ex. respectively) and *Canthidium moestum* (322 ex. and 369 ex. respectively). However, density values are extremely low and seasonal activity is restricted to a short period (October, November and December). Maximum density in both bovine and ovine droppings, has been found in November 93 (8.55 and 8.87 individuals/dung respectively), whereas mean density along the study period is 2.57 ind./dung in cattle dung and 1.53 ind./dung in sheep droppings. In sum, this preliminary study shows that populations of native dung beetles in these pastures cannot adequately remove the tons of livestock feces deposited daily throughout the country (near 11 millions head of cows). In consequence, the introduction of carefully selected species of foreign dung beetles seem to be the only alternative that could aid the native fauna in rapidly removing this dung from pasture surfaces.

10-083

ELEVATED CO₂ AND ITS EFFECT ON THE POPULATION DYNAMICS OF LEAFMINERS AND APHIDS

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The effects of an elevated CO₂ environment (200 ppm above ambient) on the population dynamics of two herbivorous insects in a model, four-trophic level, terrestrial community were investigated in the controlled environment facility, the Ecotron, at Silwood Park. Results from greenhouse studies were used to explain the findings in more detail.

For the aphid, *Brevicoryne brassicae*, increasing CO₂ levels led to earlier reproduction and an increase in population size. This response may be a consequence of an increased production of carbon based plant volatiles.

The leafminer, *Chromatomyia syngenesiae*, appeared unable to compensate for the higher C:N ratio of plants grown in elevated CO₂ environments. Marked reduction in pupal weight were recorded.

The consequences of these effects on the population dynamics of the species and their rates of parasitism are discussed.

10-084

SEASONAL ABUNDANCE OF THE NEOTROPICAL BROWN STINK BUG, *EUSCHISTUS HEROS* (HETEROPTERA: PENTATOMIDAE) IN OVERWINTERING NICHES AND BREAKUP OF DORMANCY

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The seasonal abundance of the neotropical brown stink bug, *Euschistus heros* (F.) (Heteroptera: Pentatomidae) in overwintering niches in northern Paraná state, Brazil (latitude 23° 11'S, longitude 51° 11'W) was monitored from September 1994 to August 1995. The breakup of dormancy (oligopause) was studied in the laboratory comparing the feeding activity and reproduction of adults collected in the field on different physiological condition (i.e., dormant and non-dormant). No bugs were found in overwintering niches during summer months (December to February) and during early autumn (March). From mid autumn toward winter (April-August), the number of *E. heros* captured in these niches gradually increased, decreasing thereafter with the start of spring in September. *E. heros* dormant and non dormant, taken to the laboratory at 25± 1°C, 65 ± 5% RH, and 14 HL: 10 HD photoperiod, plus suitable food (i.e., soybean pods or seeds) reassumed feeding immediately. The number of stylet sheaths deposited/day on the food was greater for non-dormant than for dormant adults. Feeding activity was greater on immature pods compared to mature seeds of soybean. Dormant females taken to suitable biotic and abiotic conditions lasted ca. 2 weeks to start reproduction.

10-086

LEAFHOPPERS (HOMOPTERA, AUCHENORRHYNCHA) OF SELECTED PEAT-BOGS IN POLAND

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The leafhoppers communities of natural highmoors of class Oxycocco—Sphagnetia in the Sudetes, Nowotarska Valley and Bieszczady Mountains in southern Poland were examined. These insects of lowmoors and transitional moors (class Scheuzerio—Caricetea) in southern and northern Poland were also considered.

Totally 136 species of leafhoppers were registered, of which two are new to Polish fauna: *Cicadella lasiocarpae* Oss. - known only from a few localities in northern part of Europe, and *Cicadula longiventris* (J. SHLB.) - a species related to taiga biome.

The leafhoppers of the examined peatbogs are characterised by the high step of heterogeneity. Variability of the species composition of leafhoppers communities of highmoors in West — East direction was observed.

In the examined material a few very interesting montane endemic and relict species were found. The characteristic species of leafhoppers communities were assigned; in most cases they were oligophagous, monophagous and higrophilous species (tyrphophiles and tyrphobiontes).

The species composition of all examined peatbogs in Poland was analysed and groups of the mutual similarities were fixed. The chorology and ecology of investigated leafhoppers were also analysed.

10-085

STUDY OF *AN. GAMBIAE* S.L. BY MARK-RELEASE-RECAPTURE IN THE VILLAGE OF BANAMBANI IN MALI.

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A capture-mark-release-recapture experiment was conducted in July in 1993 and 1994 in the village of Banambani at 25 Km northeast of Bamako in the North Sudan Savanna of Mali, to assess vector composition, population size and dispersal. The mosquitoes were captured by mouth aspirators and placed into paper cups, marked with fluorescent powders, and released at two sites: one in the center of the village and another 1Km away near the mosquito breeding sites. They were recaptured on two consecutive days following each release session. The vector population was composed of *An.gambiae* s.s (38.8% in 1993 and 76.4% in 1994) and *An.arabiensis* (61.1% in 1993 and 23.5% in 1994), which showed significant annual variations in their relative frequencies. The recapture rate for *An.gambiae* s.l. was 2.1% in 1993 and 4.2% in 1994. The mean population size of the vectors varied significantly between the two years: 10775 in 1993 and 28243 in 1994. The mosquitoes which were released in the center tended to disperse much more than those which were released near the breeding sites. In general, the mosquitoes did not disperse beyond 1 km from the release site. These results can have implications for the understanding of potential dispersal of mosquitoes from one village to another under vector control conditions.

10-087

THE EFFECTS OF CATTLE FARM SIZE AND ISOLATION ON LOCAL DUNG BEETLE COMMUNITIES

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The cattle stock and the number of cattle farms have decreased by 37 and 86 % in Finland over the past 35 years. Therefore, the habitat of specialist dung-breeding insects has become increasingly fragmented. Metapopulation models predict that species may be lost from networks of 'too' small and/or 'too' isolated habitat patches. In Finland, significant population declines have been observed in several dung beetle species (Coleoptera: Scarabaeidae: *Aphodius* and *Ontophagus*). I hypothesize that this is the result of metapopulation-level dynamics.

To evaluate the effect of farm size and isolation, I sampled the local *Aphodius* communities in individual cattle farms on the Åland islands (SW Finland). The number of specialist species breeding exclusively in the dung of domestic cattle increased with farm size (number of cows). No such effect was found for generalist species. The number of species present represents a dynamic balance between local extinctions and recolonizations. In specialist species, small cattle farm size is likely to be associated with small population sizes of beetles, and thus with a high risk of local extinction. This will shift the local species richness to a lower level.

The degree of isolation of the farm did not have any detectable effect on the number of species encountered. The lack of isolation effect is probably caused by frequent movements of dung beetles between the farms at the scale of this study. Dung beetles are strong flyers, and the patchwork of cattle farms studied is relatively dense (farms were mostly isolated by less than 4 km). Furthermore, the average local population size in dung beetles is relatively large, and hence the probability of extinction is likely to be low for most local populations. Isolation effects will probably be detected at larger spatial scales (tens of km).

10-088

THE FIRST EXPERIMENT IN LONG-TERM
PREESTIMATION OF ARMYWORMS BY MEANS OF ACCUMULATED TEMPERATURE (Digest)
--- Song Guangzhen
(Zhangqiu City Agricultural
Broadcasting School)

It's an inverse proportion in temperature with armyworm growth days($NT=K$). If the beginning growth temperature (C) and the standard error (SK) are considered into, the mathematical module must be $N=K+SK/T-(C+Sc)$. When used into the preestimation, it is to be used either in additional or divisional calculation and furthermore, there's different growing temperature in each state of worm. This makes the calculation complicated. According to the quality of screen function, with even function instead of single function, and through general calculation, I've summed up the mathematical module of the preestimation development period as follows: $K-a_1T_1^2-a_2T_2^2-.....-a_nT_n^2=0$, and upon the research of the armyworms' long-term migration flying principles, I've mapped out the programme of the armyworms' long-term preestimation: With the eggs in foreign area as the main preestimation worm state and with development period preestimation on the basis, the development tendency can be analyzed and the long-term broadcasting can be achieved.

Key words:
accumulation temperature method
armyworm
long-term preestimation

10-089

TECHNOLOGY TO PREESTIMATE
BOLLWORM EGG PEAK BY MEANS OF ACTIVE ACCUMULATED TEMPERATURE (Digest)
--- Song Guangzhen
(Zhangqiu City Agricultural Broadcasting School Shandong PRC)

Upon understanding that temperature is a molecular energetic grade in moving energy and the growth beginning temperature is the energetic grade in motivating bio-chemical reflection, as the effective accumulated temperature concept is taken away and the square of the active temperature is done, the mathematical module of the preestimate development period is founded as follows: $K-a_1T_1^2-a_2T_2^2-.....-a_nT_n^2=0$, able to be used in preestimation of appearance numbers of the bollworm egg peak in its second generation, egg peak day and egg peak velocity and as a result, the preestimation is comply with the practical estimation.

Key words:
active accumulated temperature
bollworm egg peak
preestimation

10-090

SEASONAL OCCURRENCE OF *LYGUS* BUGS (HETEROPTERA: MIRIDAE) ON FIELD CROPS IN FINLAND
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A long-term survey of *Lygus* populations on eight field crops showed that the hibernated bugs, which appeared on field crops in May, at the sowing time of spring-sown plants, first sought food and shelter from overwintered crops such as winter rye, red clover-timothy mixture, and winter turnip rape. Turnip rape and clover-timothy mixture harbored most adults until the end of June, even if some of them started to move to spring cereals (wheat, oats and barley), potato and sugarbeet after the emergence of these plants. The numbers on cereals remained small until the crops reached the heading stage. The peak occurred in the first half of August. The numbers on potato and sugarbeet were also highest in August. The total number of adults was highest on wheat. The most common species on all crops was *L. rugulipennis* Poppius, which consisted 92% of the adults.

Nymphs were found on all the studied crops. Their occurrence started in the second half of June and reached its peak from mid-July to mid-August. The numbers were highest on wheat.

10-091

PHENOLOGY AND MONITORING OF *LIRIOMYZA HUIDOBRENSIS* (DIPTERA: AGROMYZIDAE) IN POTATOES IN ISRAEL
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The pea leafminer, *Liriomyza huidobrensis* (Blanchard), was monitored using yellow sticky traps in locations in southern Israel in the spring of 1995. Initial populations were low until the second week of April, in which there was a sudden population increase in all localities. This population peak was independent of potato plant age and was not apparently related to the average daily temperature or hours of daylight.

Sampling techniques for adult leafminers were compared using yellow sticky traps and hand vacuuming. With respect to yellow sticky traps, comparisons were made between traps placed parallel and perpendicular to the ground, and at various heights above the ground in 50 cm tall potato plants. Traps placed at plant height caught the most flies with both orientations. A higher percentage of females were caught lower to the ground, presumably because there were more ovipositional sites on secondary and tertiary branches close to the ground.

Diel activity was monitored using yellow sticky traps and hand vacuum sampling in May and June in two locations (one with high and one with low leafminer populations) from 0500 to 1900. Activity patterns were identical; immediately after sunrise (0545) there was a burst of activity that declined rapidly through the morning hours as the temperature increased (to 28°C), there was no corresponding increase in activity at the end of the day as temperatures declined. Sampling activity with a hand vacuum confirmed these results under conditions of low, but not high, adult populations.

10-092

SURVEY OF THE ENTOMOLOGICAL FAUNA IN PARANÁ STATE, BRAZIL. III. SATURNIIDAE (LEPIDOPTERA)

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In addition to the Survey, Saturniidae was studied in its ecological and faunistic aspects. The material was collected using light traps installed in eight different sites of Paraná State, from October, 1986 to September, 1987. Indices of diversity and evenness (Shannon, Brillouin, Berger & Parker and α of Williams) were used to discuss richness and dominance of species. Besides using ecological indices, the data were also compared by Clustering Analysis, Principal Coordinate Analysis and Linear Correlation Coefficient. Faunistic data gathered in these sites were compared. The results were compared with a former study on Ctenuchidae (Lepidoptera).

10-093

EFFECT OF BORDER STRUCTURE ON INTERCHANGES OF EPIGEIC ARTHROPOD COMMUNITIES BETWEEN ABANDONED FIELDS AND ADJACENT WOODLANDS

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General trends in the distribution of epigeic arthropods and interchanges of Carabidae communities between formerly cultivated fields and adjacent woodlands were studied during the period July-October 1995 in five different sites on the Northern Apennines (Firenzuola, Tuscany, Central Italy). Results suggest that the structure of forest borders determines the degree of permeability to epigeic arthropods. The presence of a well-developed and closed mantel prevents grassland species from moving into the forest, whereas some woodland species (e.g. *Pterostichus micans*, *Carabus rossii*) usually penetrate into the fields. Human practices affect interchanges. Man-made tunnels across the mantel stimulate some active meadow species (e.g. *Brachynus crepitans*) to move into the forest. In young coppices, the woodland arthropod communities are characterised by a comparatively poor taxonomic structure (3 carabid species vs 7 species in mature coppices) and low active density (<1 exemplar/trap/3 days). Furthermore, some meadow species (*B. crepitans*, *Carabus violaceus*) can penetrate into such recently-disturbed forest at least up to 20 m from the border. When forest borders coincide with natural borders, such as the upper edge of a slope, interchanges between ecosystems are strongly reduced.

10-094

ECOPHYSIOLOGICAL ASPECTS OF COLD TOLERANCE OF *EPIPHYAS POSTVITTANA* WALKER (LEPIDOPTERA: TORTRICIDAE), THE LIGHT BROWN APPLE MOTH IN AUSTRALIAW. Danthanarayana¹ and H. Rohitha²Department of Zoology, University of New England, Armidale, NSW 2351, Australia¹. HortResearch, Hamilton, New Zealand²

E. postvittana can be classified as a "chill-tolerant" species. Supercooling point (SCP) varied as a function of developmental stage and geographical strain. A two-week period of winter conditioning significantly lowered SCPs. For early larval instars, winter feeding was a prerequisite for survival. Mortality occurred at sub-zero temperatures above the SCPs. The main criterion for survival - the ability of survivors to complete development and emerge as morphologically normal adults with full reproductive competence - was also determined. A proportion of larvae and pupae survived continuous exposure to sub-zero temperatures (-1 to -6°C) for periods up to 48 hours and produced viable progeny. The supercooling capacity of *E. postvittana* was not affected by season. Whilst no cryoprotective polyols were found in overwintering larvae, exposure to temperatures in the -5 to 0°C range may trigger trehalose synthesis. After exposure to overnight sub-zero temperatures for two weeks, trehalose was present as 0.4% of larval wet body weight. At such a level, trehalose is capable of preventing both freezing and desiccation damage to membranes and proteins. If increased levels of trehalose do help *E. postvittana* survive cold exposure, this may have ramifications for the disinfection of fruit leaving Australia and New Zealand. Quarantine disinfection has traditionally combined long periods of cold storage (up to three weeks) with methyl bromide fumigation. The current method of disinfection of export fruit by refrigeration at 0.55°C followed by fumigation needs to be reviewed in the light of these findings.

10-095

POPULATION DYNAMICS OF *PARNASSIUS APOLLO* (L.) IN ULUDAĞ-BURSA, TURKEY

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The population dynamics of *Parnassius apollo* was investigated in two different areas in Uludağ-Bursa between 1994 and 1995. In both areas, *Sedum album* and *Sedum pallidum* are the larval foodplants which are intermixed with nectar sources of adults. Larval population changes were examined by counts conducted weekly during the development period of larvae and the population fluctuations of adults were examined by counts conducted weekly during the flight period by capture-mark-recapture method.

Consequently, the adult flight in the first area was observed between 14 July and 8 September with a peak at the end of July, in 1994. A total of 812 individuals (45 % females) were marked and 62 of this adults were recaptured at least once. The adult flight period in the second area took place between 25 June and 1 September and 235 individuals (30 % females) were marked in total (19 butterflies recaptured). In 1995, *P. apollo* adult flights realized between 4 August and 24 September in the 1st area and between 25 July and 13 September in the 2nd area. A total of 621 individuals (37 % females) and 52 individuals (29 % females) were marked in the 1st and 2nd areas respectively. The number of butterflies recaptured at least once in the 1st and 2nd areas were 119 and 16 individuals respectively.

10-096

EVALUATION OF THE ENVIRONMENTAL IMPACT OF *Eucalyptus* CROPS ON THE ENTOMOFAUNA.

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This research deals with the environmental impact of the entomofauna on an *Eucalyptus* plantation located in Aracruz, State of Espírito Santo - Brazil. The insects were collected by using light traps in four eucalypt areas, from 1989 to 1992. Through the faunistic analysis the predominant species were selected to be used as ecological indicators which, together with the diversity and physiography indexes, the quotient and percentage of similarity and the non parametric tests of Kruskal-Wallis and Wilcoxon, have permitted the evaluation of such impact. The results were as follows: the mean diversity index of the region indicated a high richness of insect species in the area; there was a predominance of beneficial insects in relation to eucalypt pests; the diversity was dependent on the physiography index of the areas; one of the areas preserves the faunistic conditions close to those of a natural forest. The influence of the eucalypt areas on the entomofauna was low; when the eucalypt trees were cut it was observed a decrease in the number of insect pests in the area, but the faunistic effect was not significant; the environmental impact of the eucalypt plantation on the entomofauna was very low and did not interfere with the ecosystem.

10-098

DOES THE NATURE OF WOODLAND HABITATS AFFECT THE MOVEMENTS OF *ABAX ATER* VILL. (COL., CARABIDAE) IN A HEDGEROW NETWORK LANDSCAPE: A RADIOTRACING STUDY

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Abax ater is a forest beetle which was shown to survive in bocage landscapes as metapopulations. Local populations are located in woods, lanes and nodes of the network whereas some linear wooded habitats can be used as corridors, providing paths for recolonization. The aim of our study was to assess whether the nature of woodland habitats in a bocage influence movements of *Abax ater* and thus its potential dispersal abilities.

The walking pattern and intensity of movements of the species were examined during two months using a radiotracing method in a wood, a lane, a hedgerow with continuous tree and shrub layer and a hedgerow characterised by numerous gaps in the vegetation cover. Although the walking pattern remains similar in the four habitats as soon as individuals do not enter adjacent cultivated parcels, the intensity of movements significantly differs among habitats. Mean distances covered and total space occupied during the study were the highest in the wood and progressively decrease as vegetation cover in the linear elements decreases. In linear elements, many individuals left the habitat and got lost in adjacent fields where most of them died. On the opposite, none of the individuals traced in the wood died or left the habitat.

This study indicates that the diffusion of *Abax ater* is influenced by the nature of woodland habitats. It also shows that the quality of linear features, especially vegetation cover, affects their efficiency as dispersal corridor so that attention should be paid to their management in order to maintain functional links between local populations.

10-097

INFLUENCE OF WILD CRUCIFERS ON BIOLOGY AND FLIGHT ABILITY OF THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: YPONOMEUTIDAE).

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The larvae of *P. xylostella* were fed on five weed crucifers and crop, cabbage. The life history traits and flight activity of adults were measured. The developmental period of larvae which were fed on Indian marshcress (*Rorippa indica*) and Virginia pepperweed (*Lepidium virginicum*) was shorter than those fed on cabbage but the former larvae emerged as smaller adults. The female adults fed on weed crucifers had less fecundity than those fed on cabbage but the former had a higher flight activity. A clear trade-off was observed between fecundity and flight activity.

In the comparison of individuals which were fed on the same host plant, large sized females laid more eggs and also had a higher flight activity than the smaller one. The larger adults which flew more had larger flight

10-099

BODY SIZE, SEXUAL RECEPTIVITY AND LARVAL CANNIBALISM IN RELATION TO PROTANDRY AMONG *TOXORHYNCHITES* MOSQUITOES

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Males of *Toxorhynchites rutilus* and *T. amboinensis* emerge as adults significantly sooner than females, the difference between sexes more pronounced in the latter species. In contrast to other protandrous mosquitoes, male and female *T. rutilus* did not differ in mean body mass, and *T. amboinensis* females are only slightly larger than males. Protandry in the context of size similarity of the sexes is maintained by significantly faster growth of males. Sexual maturity is reached in both sexes approximately one day after adult eclosion, which occurs primarily in the late afternoon. Predatory fourth instar larvae of *T. rutilus* that co-occurred in treeholes were far more similar in body mass than individuals of that same stage that occupied different treeholes. In surrogate treeholes set experimentally in nature, the rate of cannibalism was significantly higher among *T. rutilus* fourth instars that differed in body mass. Presumably, the size similarity that minimizes larval cannibalism selects against sexual size dimorphism in adults of these species.

10-100

SPATIAL DISTRIBUTION OF *TETRANYCHUS URTICAE* KOCH (ACARI:TETRANYCHIDAE) ASSOCIATED WITH ITS PARENTAL LIFE TYPE AND EGG-LAYING BEHAVIOUR

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Aggregative spatial distribution of population of *Tetranychus urticae* Koch (TSM) on carambola orchard was evaluated by index of dispersion (ID), Green's coefficient of dispersion (Cx), mean crowding index (me), patchiness index (me/m), Morisita's coefficient of dispersion (I_{δ}, I_{β}), Taylor's power law and Iwao's patchiness regression (α, β). The magnitude of fluctuation and increment of the indices values of ID, me, and me/m were highly correlated with those of the magnitude of changes and increments of the densities of the TSM. The results of estimated slopes from the regression analyses of variances over means on the TSM populations by Taylor's power law model are all larger than 1. All different stages of the TSM populations are therefore assumed to be the aggregative ones. Iwao's patchiness regression analyses on TSM populations shows TSM population forms a high aggregation within and among the patches. Very high degree of aggregation and patchiness of egg and larval populations of the TSM are inferred as the results of female life type and egg-laying behaviour and pattern within the patches.

10-102

RELATIONSHIPS BETWEEN SUPERCOOLING POINT AND COLD HARDINESS OF THE CLOVER LEAF WEEVIL, *HYPERA PUNCTATA* (COLEOPTERA: CURCULIONIDAE) IN JAPAN

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Hypera punctata overwinter as any developmental stage other than pupae in Tsukuba, Japan. They appear to be able to grow continuously during winter. The lower thermal threshold of larval development is below 10°C. The proportion of late instar larvae in the population increased gradually between December and May, and 4th (last) instar larvae were found in February onwards. The supercooling point (SCP), often used as an indicator of cold hardiness, of eggs was the lowest (-22 to 27 °C) among all developmental stages. In larval stages, there was a significant positive correlation between the SCP and larval body weight. Under starving conditions, the correlation became higher, and the SCP decreased by 5 to 7°C. A similar correlation was also found for each larval instar except for the 4th instar. In the 3rd and 4th instars, most larvae exposed to -5 or -10°C survived if they were not frozen, but died if frozen. On the other hand, the prefreeze mortality (mortality without freezing) at -10°C was high in the 1st and 2nd instars. It was higher in starved larvae than in fed ones. All 1st instar larvae did not survive an exposure to -15°C, though many larvae had not been frozen. These results suggest that the SCP is not always a good indicator of cold hardiness in this beetle.

10-101

SPATIAL DISTRIBUTION PATTERNS OF *AMBLYSEIUS OVALIS* (EVANS) (ACARI: PHYTOSEIIDAE) ASSOCIATED WITH ITS PREY DISTRIBUTION PATTERN AND EGG-LAYING BEHAVIOUR

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The spatial distribution patterns of *Amblyseius ovalis* (Evans) on carambola orchard was evaluated by index of dispersion (ID), Green's coefficient of dispersion (Cx), mean crowding index (me), patchiness index (me/m), Morisita's coefficient of dispersion (I_{δ}, I_{β}), Taylor's power law and Iwao's patchiness regression (α, β). The behaviour of aggregation of *A. ovalis* was positively correlated with the initial incremental phase of density and aggregativeness of its prey, *Tetranychus urticae* Koch. The aggregative distribution character of *A. ovalis* found on larvae but not on the other stages' populations were demonstrated by Taylor's power law model. The egg and larval quadrat were formed by a single individual, and the degree of incremental chances with another individual in the same quadrat for nymphs, females and males, were inferred by the estimated α and β values from Iwao's patchiness regression analyses. The behaviour of one-egg-deposition following an emigration performed by females in the quadrat was also demonstrated by their egg-laying pattern at low prey density.

10-103

EFFECTS OF POPULATION AND HABITAT PARAMETERS ON MOVEMENT PATTERNS IN GRASSHOPPERS (CAELIFERA: ACRIDIDAE)

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The activity patterns of four grasshopper species at eight study sites in Thuringia/Germany were investigated in 1993 and 1994 using several individual marking techniques. Owing to an additional marking of each individual by reflective tape, consecutive nocturnal observations could be conducted. Altogether more than 8.000 observation points of about 2.100 marked adults of *Stenobothrus lineatus* (PANZER), *Oedipoda germanica* (LATR.), *Gomphocerus rufus* (L.) and *Chorthippus parallelus* (ZETT.) were analysed by three main parameters of individual movement: mean daily movement, dispersal range and home range. The results of the study indicate a dependence of the activity patterns on size and shape of the habitat as well as on the population density. Furthermore, broadly scattered distributions occurred due to differences between individuals, sexes, and in the individual life span.

10-104

INSECT POPULATION VULNERABILITY ANALYSIS (PVA): CASE STUDIES IN GRASSHOPPERS AND BUSHCRICKETS

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An approach of a grasshopper population vulnerability analysis (PVA) in fragmented landscape units is demonstrated in this video film. In two study sites near Jena /Thuringia (Germany), a semi-arid grassland and a limestone quarry, demography, movement patterns and habitat requirements of *Oedipoda germanica* (LATR.), *Stenobothrus lineatus* (PANZER) and *Phaneroptera falcata* (PODA) were studied.

The videofilm introduces in the procedure of a PVA and shows methods and techniques used in field and laboratory experiments, like measurements of vegetation structure, microclimate and insect body temperature as well as registration of nymphs with biocoenometer, individual marking of adults, night observations with head-lamps and weighing of females for the determination of oviposition. With regard to minimum viable populations, some results are mentioned.

10-105

A POPULATION VULNERABILITY ANALYSIS OF THE RED-WINGED GRASSHOPPER, *OEDIPODA GERMANICA* (LATR. 1804) (SALTATORIA: ACRIDIDAE)

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The xerophilous Red-winged grasshopper, *Oedipoda germanica* (LATR.) is threatened by extinction in Germany because of a drastically decline of suitable habitats in the past. In a limestone quarry near Jena (Thuringia, Germany), which is situated at the northern area boundary of this species in Central Europe, exists one of the last populations in Thuringia. In a field study from 1993-1995 population dynamics and movement patterns of *Oedipoda germanica* were investigated as well as reproduction parameters in greenhouse experiments. Using these data an individual based model was developed to estimate the minimum viable population size (MVP) and survival rate probability of the population.

10-106

HEAVY METALS IN CARABIDS (COLEOPTERA: CARABIDAE)

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Investigations of invertebrate roadside communities, polluted by heavy metals, started in 1983, showed, that in general the ability to accumulate heavy metals followed the order: annelids (earthworms) (the best accumulators), isopods, spiders, holometabolic insects (carabids, fly larvae) (the worst accumulators). The observed differences were connected with the existence of different life strategies as related to preadaptations to heavy metal stress in different groups of animals (Butovsky, 1993). More detailed investigations on heavy metal contents in 15 carabid species, collected in roadside ecosystems of Russia and the Netherlands, showed that Russian carabids contained Cu (13.1-26.8 ppm) and Zn (56.4-184.8 ppm) and didn't accumulate the most toxic Pb or Cd. In Dutch carabids we found Cu (5.9-30.1 ppm), Zn (27.1-118.6 ppm), Pb (0-13.0 ppm) and Cd (0-1.9 ppm). In all studies heavy metal contents in carabids were not related to the distances from motorways. In Russian samples we found species-specific and sex-specific differences in accumulation of Zn and Cu. Females in most cases contained more Zn than males. We couldn't find any differences in heavy metal contents between myxophagous and zoophagous species. In most cases the correlations between relative abundance of dominant carabid species *P. cupreus* and *P. melanarius* didn't occur. However in these species we found the decrease of heavy metal contents by the end of the field season (in September). The absence of expressed differences in heavy metal concentrations between species, sexes and trophic groups was connected with (1) similar spectrum of nutrition and life strategies; and (2) high ability to excrete heavy metals. These circumstances reduce the possibilities to use carabids as indicators of environmental quality in ecosystems, polluted by heavy metals.

10-107

LARVAL DEVELOPMENT OF *BIBIO* SPECIES (DIPTERA, BIBIONIDAE) AT HIGH ALTITUDES IN NORWAY

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The life cycles of the bibionids *Bibio rufipes* (Zetterstedt) and *B. siebkei* Mik from the lower alpine zone in Southern Norway were investigated. The development goes through seven instars, and takes two years, with the larvae overwintering in the fourth and seventh instar. Imagines eclose in July and August. Dry weight of *B. rufipes* larvae in their second summer increases rather rapidly between approximately 20 June and early August while there is no increase between October and June. Dry weight declines to approximately half its October value in spring, but this is probably because larvae collected in spring have no gut content.

Material from one season suggests that development in the larger species *Bibio pomonae* (Fabricius) takes three-four years in the lower alpine zone.

10-108

THE GROUND BEETLE COENOSIS (COLEOPTERA: CARABIDAE) OF THE RIPARIAN WOOD OF PANFILIA (PADANE PLAIN, NORTHERN ITALY)

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A three years research (1993-1995) on the ground beetle communities has been carried out at the Wood of Panfilia by using different methods (pitfall trapping, direct catching, sweep net, beating tray and soil sifting). The Panfilia Wood, originated about two hundred years ago and covering about 80 hectares, is an interesting biotope on the right bank of the Reno River both for the rarity of woodlands in Padane Plain and for the peculiarity of its phytocoenosis. The botanic association described as *Carici-Fraxinetum angustifoliae*, comparable to those present in Central-Southern Italy, is nowadays considered more similar to the alluvial plain forest type of hard wood (*Fraxino-Ulmetum* and *Ulmo-Quercetum*). The wood is bounded from the adjacent plain by a high embankment and the seasonal floods coming from the river, with the consequent frequent mud sedimentation, are one of the most important ecological factors subsisting on the biotope. The carabid assemblage, with a total of 138 species, was preliminarily analyzed from a qualitative and quantitative point of view, some diversity indices were calculated and some faunal, ecological and zoogeographical notes were reported. The most important sampled species were: *Lasiotrechus discus* (F.), *Atramus collaris* (Ménétriés), *Europhilus micans* (Nicolai), *Scybalicus oblongiusculus* (Dejean), *Parophonus planicollis* (Dejean) and *Polystichus connexus* (Fourcroy) in the riparian *Salicetum*; *Carabus granulatus interstitialis* Duftschmid, *Anillus florentinus* Dieck and *Phonias ovoideus mainardii* (Straneo) in the hard wood forest; *Cylindera germanica* (L.), *Campalita auropunctata* (Herbst) and *Leistus ferrugineus* (L.) in the alluvial terrace meadow; *Amara concinna* Zimmermann, *Anisodactylus intermedius* Dejean, *Acinopus picipes* (Olivier) and *Microderes scaritides* (Sturm) on the embankments and six species of Lebiini were found on the herbaceous plants and on the foliage of shrubs and trees.

10-110

OVIPOSITION DECISIONS BY A TREE-HOLE MOSQUITO: A TEST OF MULTIPLE HYPOTHESES IN THE FIELD.
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This field study tested hypotheses about egg-laying decisions by a tree-hole mosquito, *Aedes triseriatus* (Say). We predicted that *A. triseriatus* would exhibit choosy oviposition behavior because of their dependence on restricted, sometimes limited, resources and their potential exposure to intraspecific larval competition. We placed artificial microcosms, stocked with tree-hole water and lined with paper oviposition substrates, in a woodland. During two field seasons, we examined oviposition rates in response to larval density and to number of eggs already deposited at the site. First, females were significantly attracted by a high number of eggs. On the other hand, the response to larval density was variable. During one season (1992), females avoided higher densities early in the season, but responded positively to that factor when they laid eggs destined for diapause, later in the season. The second field season (1994) did not yield a significant response to larval density. A third factor tested in 1992, presence of predatory mosquito larvae (*Anopheles barberi*), elicited no detectable response by egg-laying *Aedes*. Our findings suggest that females may have been selected to find suitable habitats without regard to larval density. We speculate that this may be so because their offspring have a particularly relevant adaptation—pharate larvae detect cues that reflect larval density and can regulate their hatching in response, emerging when the potential for interspecific competition is low. The positive response to eggs by ovipositing females is more difficult to understand and was not expected based on findings by other investigators.

10-109

CECIDOMYIIDAE (DIPTERA) ASSOCIATED WITH MYRTACEAE AT RESTINGA OF BARRA DE MARICÁ, RIO DE JANEIRO
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The Restinga of Barra de Maricá (RJ) shows a great richness of higher plants (195 species belonging to 70 families, according to ARAÚJO & HENRIQUES, 1984). Among these families, Myrtaceae are represented by 11 species and show the greatest variety of Cecidomyiidae galls: 14 kinds on 6 species of host-plant (2 kinds on *Eugenia copacabanensis* Kiaersch, 3 on *E. rotundifolia* Casar, 2 on *E. uniflora* Linnaeus, 1 on *Myrcia ovata* Cambessedes, 2 on *Myrciaria floribunda* (Camb.) Legrand and 4 on *Neomitranthes obscura* (DC.) Legrand. The galls were collected from 1986 to 1994. Cecidogenous, inquiline and predators species were reared and identified in laboratory. *Stephomyia tetralopae*, *S. espiralis* and *Trotteria* sp. were found on *E. copacabanensis*; *S. rotundifoliorum*, *Lestodiplosis* sp., *Dasineura* sp., *Resseliella* sp. and one Cecidomyiidi on *E. rotundifolia*; *Neolasioptera eugeniae* and one Cecidomyiidi on *E. uniflora*; *Myrciariamya bivalva* and *Dasineura* sp. on *M. floribunda*; n. gen., and n. sp. on *Myrcia ovata*; *S. mina*, *Dasineura* sp., n. gen. and n. sp., one *Clinodiplosini* and one Cecidomyiinae on *N. obscura*. Galls were characterized by the shape (fusiform, cylindrical, elliptical, conical, spheroid, spiralled, marginal leaf roll, bivalve or pine cone shaped), color (green, red, yellow or brown), number of internal chambers (1 or 0) and plant part on which the gall is situated (leaf, stem, axillary or apical buds). Data on number of larva per gall and the period of galls occurrence are given. The material was prepared according to GAGNÉ, 1994 and is deposited in the Diptera collection of Museu Nacional.

10-111

MALE LARVAL NUTRITION INFLUENCES THE REPRODUCTIVE SUCCESS OF BOTH SEXES IN *CHORISTONEURA FUMIFERANA*
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Work on the polyphagous species *Choristoneura rosaceana* demonstrated that while different larval host plants did not affect the proportion of virgin males of acquiring a mate, it had a significant impact on their ability to remate. Furthermore, the total fecundity and fertility of females mated with males reared on poor quality hosts (i.e. hazelnut) was significantly lower than those mated with males fed on high quality hosts (i.e. striped maple). A parallel study was undertaken to examine the effect of larval food quality in a related, eruptive oligophagous species, *C. fumiferana*. The reproductive success of both sexes was compared when males were reared on the same host species (balsam fir) but varying the foliage quality, to reflect endemic and epidemic phases in the population cycle. Females mated with males reared on high quality foliage were more likely to remain refractory following first mating. Furthermore, the reproductive success of females mated with males reared on poor larval food were significantly lower than for conspecifics mated with males provided high quality food during larval development. These results will be discussed in relation with possible physiological mechanisms regulating reproduction and migration in *C. fumiferana*.

10-112

THE INFLUENCE OF MORPHOLOGICAL DIFFERENCES BETWEEN APHID MUMMIES ON PREDATION BY VARIOUS TYPES OF INVERTEBRATE PREDATORS

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Field observations indicated that aphid parasitoid cocoons are vulnerable to several species of invertebrate predators. Unexpectedly high levels of predation (>85%) were observed in both potato and alfalfa plots. It is hypothesized that *Praon* cocoons, spun underneath the mummified aphid, are less susceptible to predation than *Aphidius* cocoons, spun within the mummified aphid. In the laboratory, I measured the ability of mandibulate (coccinellidae, chrysopidae) and piercing/sucking predators (miridae) to detect and feed on *Praon* and *Aphidius* cocoons. In most cases, no clear patterns were evident. However, some experiments indicated that the physical traits of *Praon*-type mummies provide additional protection against fourth instar and adult coccinellids to the developing parasitoids. In this instance, the aphid skin seems to divert predators from attacking the parasitoid cocoon underneath.

10-114

POSSIBLE DISSEMINATION OF *SPHAEROPSIS SAPINIA* (FR.) DYKO ET SUTTON F.SP. *CUPRESSUS* SOLEL ET AL. (DEUTEROMYCOTINA) BY PSOCOPTERA PSOCOMORPHA IN ITALY

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Due to their alimentary interest in conidia of plant pathogenic fungi, Psocoptera are suspected to be vectors of plant diseases (DE MARZO et al., 1994). Further evidence of this matter arises from the study of some species of Psocoptera, which commonly occur on the canopy of many Cupressaceae and Pinaceae in Southern Italy; in fact as two of them, *Ectopsocus briggsi* McLach. (Ectopsocidae) and *Caecilius burmeisteri* Brauer (Caeciliidae), were recognized as able to disseminate living conidia of *Sphaeropsis* by their excrements.

Conidia in the alimentary canal were observed by dissecting several specimens of Psocoptera, collected in different localities in Apulia and Basilicata on branches of *Cupressus sempervirens* and *C. arizonica*. *Sphaeropsis* conidia were present together with those of other genera: *Alternaria*, *Cladosporium*, *Leptosphaeria*, *Mucor*, *Pestalotia*, *Phomopsis*, *Seiridium* and *Stemphylium*.

Survival of the ingested *Sphaeropsis* conidia was evaluated by plating some specimens of Psocoptera in APS medium in Petri dishes. Previously the specimens were washed by sterile water and dried up with sterile blotting-paper. Growth of *Sphaeropsis* mycelium was recognized, together with that of other forms of fungi, up to 10 days in dishes incubated at 25°C.

After 30 days mature picnidia were found and were identified as *Phaeropsis sapinea* on the basis of morphometric data. This strain was used for trials of pathogenicity on *Cupressus* and showed symptoms of disease after 30 days. Biological and morphological characters let us to identify this strain as *cupressi* Solel et al.

References

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10-113

POPULATION REGULATION OF A FOREST-FLOOR WOLF SPIDER: RELATIVE IMPORTANCE OF FOOD LIMITATION AND MORTALITY FROM ARTHROPOD AND VERTEBRATE PREDATORS

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We investigated the relative contributions of bottom-up and top-down control processes to population regulation of *Schizocosa*, an abundant wolf spider in the leaf-litter community of deciduous forests in eastern North America.

We increased densities of Collembola, mites and adult Diptera in ten 2 x 5 m open plots by periodically adding mushrooms, potatoes and instant *Drosophila* culture medium for 2.5 months. In comparison to control plots, densities of *Schizocosa* doubled; individual spiders also were larger. In a second experiment, we excluded birds and removed rodents, lizards and amphibians from five fenced 50-m² plots for 1.3 years. *Schizocosa* densities did not differ between the vertebrate-exclusion plots and five open reference areas. To examine possible top-down control by arthropod natural enemies, we reduced densities of centipedes, predacious beetles, and all spiders other than *Schizocosa* in six fenced 4-m² plots for 2.5 months. *Schizocosa* mortality was high (70%), but did not differ between predator-reduction and control treatments.

These results reveal that increased productivity of prey has a greater impact on population density of *Schizocosa* than does reduced mortality from arthropod and vertebrate predators.

10-115

RICHNESS AND DIVERSITY IN THE WOODLAND SOIL FAUNA IN RELATION TO DIFFERENT DISTURBANCES

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Six woody areas of western Sicily (central Mediterranean), between 800 and 1000 m a.s.l., were selected; they were characterized as follows: a) and b) mature woods dominated by *Quercus pubescens* and *Q. ilex*, the first one undisturbed, the second burnt in 1994; c) mature wood of *Q. pubescens* and *Ilex aquifolium* extensively grazed by cattle; d) as c, but without grazing and cleared of the understorey; e) mature wood dominated by *Q. ilex*, with a rich understorey; f) reafforestation of conifers, c. 30 years old.

In 1995 cores of soil were seasonally extracted in four different randomly chosen locations of each woody area; soil fauna was counted after a 7-days extraction by a Tullgren Funnels.

Undisturbed woodlands showed both the highest richness and taxon diversity. Fire depleted richness, and as probable consequence of prey biomass decrease, predators (e.g. Acari) were mostly numerically reduced; species active on the soil surface and living within the litter, particularly decomposers, also decreased after the fire passage. Grazing had an evident impact on richness and diversity in comparison with undisturbed woodlands; this agrees with the results from other mediterranean areas. Eradication of understorey possibly caused the availability diminution of the organic matter in the soil and thus the richness of detritivores, which in turn affected that of predators. Finally values of conifer reforestation show the poorness of this artificial habitat and suggest the need of long-term manage in order to transform it in a mixed woodland.

Total richness and taxon diversity showed a spring-summer peak; herbivores, mostly dependent on the growth rate of plants, were more abundant during the peak of growth season; summer drought and heat, reducing water availability, were probably responsible of seasonal variation of populations. Organic matter influenced the abundance of species, particularly detritivorous ones, and the spatial structure of the community. Predators, generally used as good bioindicators because are dependent on prey abundance, indeed resulted to be more abundant in the undisturbed woodland in respect to those affected by different types of disturbances.

10-116

STAPHYLINIDAE (INSECTA: COLEOPTERA) SOIL COMMUNITIES OF WOODLAND REMNANTS IN EASTERN SICILY

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The Staphylinidae community structure of the woodland remnants, that escaped the extensive deforestation of the last three centuries, were studied. The examined remnants are located on the Peloritani Mountains (on Oligocene-Miocene flysch), on Mount Etna (on volcanic substrata), and on the Iblei Mountains (on limestone). The structure of the remnant communities was compared with that of the communities of the Nebrodi Mountains woodlands (on sandstone and flysch), the largest forest system of the island. Quantitative data were obtained by pitfall-trap samplings carried out monthly for a year. Correspondence analysis was utilised for comparing the community structure. Despite a considerable invasion of open land species, the communities of the forest fragments generally maintain most of the nemoral species found in the large Nebrodi's woodlands. To explain the differences found in the community structures, the following factors were considered: the climate, typology and frequency of natural and anthropic disturbances, size and heterogeneity of the remnants, and the characteristics of the rocky substrata.

10-118

THE BEHAVIOURAL PECULIARITIES OF CONSUMERS OF DIFFERENT TROPHIC LEVELS IN THE SYSTEM "PLANT-PHYTOPHAGAN-ENTOMOPHAGAN".

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The interrelations within the tritrophic systems were investigated in model experiments by the example of *Myzus persicae* Sulz., *Aphis gossypii* Jlov., *Rhopalosiphon padi* L. (Aphididae) and *Aphidoletes aphidimyza* Rol.(Cecidomyiidae) at a period of plant invasion with the insects. A number of cultivars of russian turnip, cucumber, wheat and maize differing in their morphological and physiological properties has been used in the investigation. The diversity of studied insect species and plant forms makes it possible to reveal the following regularities. Orientation of aphids and *A.aphidimyza* may be similar at the stage of distant search and differ at the stage of contact choice of plant. The host plant always modifies aphid behavioural responses and physiological processes such as selectivity, development, social behaviour, migration ability and metabolism. The behaviour of *A.aphidimyza* adults is conditioned by the presence of honeydew on plants while the presence of aphids themselves is not necessary for oviposition. Honeydew is a food source for *A.aphidimyza*. The choice of oviposition sites by females is determined by honeydew quality (composition and physical condition) and localization on plant. The properties of honeydew are influenced by both host plant (phenotype and metabolism) and aphids metabolism, behaviour, topical specificity and population structure). It can be concluded that the complicated interactions take place at first stages of formation of tritrophic system. These interactions are based on trophic relations and can be defined as commensalism. Good agreement in search behaviour between the theaphids and the entomophagan is mainly determined by similarity of their requirements to the conditions which are created on plant surface. Aphid honeydew is operating as key chain which enables all the system begin to function as tritrophic one.

10-117

REPRODUCTIVE PERFORMANCE IN A NEOTROPICAL GRASSHOPPER (ORTHOPTERA: ACRIDIDAE: GOMPHOCERINAE)

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Rhammatocerus conspersus (Bruner) (R.c.) lives in open grassland habitats in south Brazil, Argentina and Uruguay. It is a polyphagous species but preferably feeds on grasses; occasionally it attains pest status in native pasturelands in Brazil. R.c. is a univoltine species with an 8 months long obligatory egg diapause. Under laboratory conditions maturation of the ovaries is incomplete and fecundity is negligible at the average air temperature (25°C) of Summer months. With a radiant heat source (35-38°C) R.c. attains a body temperature well above the air Summer average maximum (30°C); females are long lived (\bar{x} 150 days), the pre-oviposition period is comparatively short (\bar{x} 24 days), egg-laying extends for nearly 3 months and potential fecundity is high (\bar{x} 724 eggs/female). Under field conditions females die young, before the onset of Autumn. It is suggested that in subtropical Brazil (29°S 55°W), egg diapause provides the means to synchronize the active, basking stages with the months of maximal temperatures and insolation.

10-119

OBSERVATIONS ON THE HYDRADEPHAGAN COMMUNITY (COLEOPTERA: HALIPLIDAE, DYTISCIDAE) OF A DAMP AREA IN THE "PIANURA PADANA".

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The fresh water damp areas of the "Pianura Padana" are shown to have a limited extension; actually they are part of a territory which is widely exploited for agricultural purposes. Our sampling site, "Ercolana", is a damp area of about 60 hectares, created in 1990, at Malalbergo (Bologna, South-East of the "Pianura Padana") by conversion of agricultural lands to an area suitable for shooting and fishing. The wetlands of "Ercolana" consist of two marshes extending for 28 hectares and a damp meadow of 8 hectares which is subject to periodic flooding. From April to December 1995, a total of 2117 adult specimens of 26 species of Hydroadephaga were collected from different sampling sites of the "Ercolana". The taxonomic composition of the hydradephagan community existing in the study area has been analyzed, together with the main ecological and biogeographical traits of the species found: certain of these species (e. g. *Hydroporus planus* (F.), *H. nemnonius* Nicol. and *Ilybius subaeneus* Er.) were seen to be particularly frequent. The data collected during the present investigation was compared with the information available in literature concerning the occurrence of Hydroadephaga in other similar habitats of northern Italy. Furthermore, the index of Dice-Sørensen shows that the hydradephagan fauna of the study area has the best affinity with that of the typical pools of the "Pianura Padana". The present study was undertaken in order to assemble data concerning the presence and distribution of this group of aquatic insects of the "Ercolana". The results of hydradephagan community composition in the study area, as well as a comparison with similar habitats for this group of insect elsewhere in northern Italy, will be reported.

10-120

EGG PRODUCTION AND RESORPTION IN A PARASITIC WASP: CAN FEMALES PREDICT FUTURE EGG NEEDS?

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The number of eggs available for oviposition inside the female's ovarioles (or *eggload*) is a crucial determinant of parasitoid reproductive behaviour. In some species of parasitoids female eggload is determined by three processes: egg production, egg resorption and egg laying. The resulting complex dynamic nature of the ovaries of these parasitoids has hampered the incorporation of realistic time-dependent variation in egg load into models of parasitoid behaviour that allow the physiological state of the parasitoid to change with time.

Egg resorption is a specific type of reproductive strategy in which oocytes in the female's ovarioles degenerate instead of being laid as eggs. It is often assumed to be a means of recycling eggs that have reached maturity but have not been required for oviposition; or a means of obtaining nutrients at times of stress. We study whether the number of mature eggs carried by the parasitoid and the rate of egg resorption can be adjusted in environments of different quality where the parasitoid can expect to encounter different numbers of hosts. We predict that wasps exposed to more profitable conditions will maintain a larger reserve of mature eggs, in expectation of greater oviposition opportunities. We further predict that this adjustment in egg load will be effected by a reduction in the rate of egg resorption rather than by an increase in egg production, since it is a less wasteful way of dealing with limited resources. We carried out a laboratory experiment to test the ability of female *Leptomastix dactylopii* (Hym: Encyrtidae) to adjust its egg load in response to environments of different quality. Wasps in the more profitable treatments were able to compensate for the increased number of eggs laid in the hosts, through an increase in their egg production rate, but were not able to predict increased future egg needs.

10-121

RESPONSE OF SPIDER MITES TO ODOURS FROM PLANTS DAMAGED BY HERBIVORES

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Plants produce volatiles in response to herbivore attack. These volatiles attract natural enemies of the herbivores. However, it is still unknown how herbivores respond to plants damaged by herbivores.

In Y-tube olfactometer experiments and release-recapture experiments in a greenhouse, we tested the response of *Tetranychus urticae* when offered a choice between clean cucumber plants and cucumber plants infested with conspecifics or western flower thrips.

We found a slight but significant preference for plants infested with conspecifics over clean plants, and a strong and significant preference for clean plants over plants infested with western flower thrips.

By avoiding plants with thrips, spider mites do not only avoid competition for the host plant, but also intraguild predation by thrips. Therefore, this kind of complex trophic interaction can also influence the pest status and the success of biological control of spider mites and thrips in crops where they occur together.

10-122

THE EFFECTS OF NUMBER, QUALITY, AND POSITION OF RESOURCES WITHIN SUCROSE PATCHES ON THE FORAGING BEHAVIOR OF THE FRUIT FLY *DROSOPHILA MELANOGASTER* (DIPTERA: DROSOPHILIDAE)

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Fruit flies *Drosophila melanogaster* were allowed to forage on two patches containing wells filled with sucrose solution. We tested the effects of varying sucrose concentration (resource quality), number of wells filled (number of resources), and position of the resources on the foraging behavior of the flies.

All variables affected patch preference as well as feeding frequency. The flies were able to select patches with higher concentration of sucrose, larger numbers of resources, and larger resource density.

10-123

THE INSECT FAUNA OF ROME: SOME CONSIDERATIONS ON THE RESULTS OF A FAUNISTICAL SYNTHESIS

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Rome, Italy's largest city, has about 3 million inhabitants and covers an urban surface area of 380 km². The insect fauna of this area has been object of research since the second half of the 19th century. Rome is one of those rare cases in Europe where urban expansion in the last century took place on an area, the "Campagna Romana", which had been practically disinhabited. A recent study edited by the Author based on a critical revision of the entomological literature published from 1880 to nowadays together with other unpublished material has evidenced the presence and the microdistribution of about 5000 species belonging to not less than 300 families. Such information, although so vast, is however still incomplete. Documentation for groups such as Heteroptera, Coleoptera, Diptera, Macrolepidoptera and Hymenoptera Aculeata, which have traditionally been studied by local entomologists is quite complete but groups such as Collembola, Psocoptera, Phthiraptera, Thysanoptera and Homoptera are still poorly documented. From a preliminary global analysis of data collected, it can be clearly seen that from the end of the 40's there has been a general impoverishment of the species in particular in those communities linked to wetlands. The theoretical and applicative importance of a study on the entomofauna of Rome (microdistribution of the species in relation to bioclimatic factors, research on isolated populations, utilization of the information in environmental planning, pest control) is highlighted and at the same time certain aspects linked to urbanization which have influenced or influence the insect fauna are evidenced: 1) environmental modifications which took place in the last hundred years on a local scale (the rapid urbanization of the city from 1870 onwards due to its role as capital of Italy) and on a regional scale (land reclamation and progressive human impact on the "Campagna Romana", modifications to the flow of the River Tiber and its tributaries, chemical control of culicids as vectors of malaria); 2) the presence of large central green areas and greenways (historical villas and archaeological areas) and their role in the conservation of biodiversity.

10-124

MOVEMENT OF THE TWO WEEVIL PESTS OF SWEET POTATO, *CYLAS FORMICARIUS* AND *EUSCEPES POSTFASCIATUS* (COLEOPTERA: BRENTIDAE-CURCULIONIDAE) MEASURED BY COMMERCIALY AVAILABLE ELECTRONIC DEVICES

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In the Ryukyu archipelago, the southernmost part of Japan, *Cylas formicarius* and *Euscepes postfasciatus* are the most destructive exotic pests of the sweet potato. Attempts to eradicate them by using a sterile insect technique and a male annihilation method, respectively, are underway in Okinawa Prefecture. It is essential to assess their dispersal ability for the success of the eradication project. Therefore, we measured adult locomotion behavior of the two weevils and the flight activity of *C. formicarius* (*E. postfasciatus* has no ability to fly) using an actograph, a flight mill system and a newly developed tracking system in the laboratory.

Actograph: No clear difference was detected in the incidence of walking in the sexes of *C. formicarius*, however, aged female adults of *E. postfasciatus* moved more actively than males.

Flight mill: It is quite evident that *C. formicarius* males flew much longer than females. But these values, far less than 50 m on average during 24 hours, were very small compared with those of other insects and nearly 95% of the females tested never flew at all. Since these two systems were manually operated, they were improved by the introduction of computer-controlled devices. The new system revealed that the two weevils moved mainly in dark periods irrespective of sex.

Tracking system: A color image processing unit (Color tracker) combined with a computer enabled us to track adult locomotion of 16 individuals at an interval of one second simultaneously under a constant light condition. Analyzing the locomotion data obtained by this system, relative data by the actograph (number of counts) could be transformed exactly into absolute data (total distance).

10-126

INTERACTIONS BETWEEN ENDEMIC HERBIVOROUS INSECTS AND AN INVADDED WEED *SOLANUM CAROLINENSE* L.

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A perennial weed *Solanum carolinense* L. invaded from North America into Japan is spreading over agricultural fields, grass lands, and lawn and abandoned areas. The weed provides endemic herbivores with a novel opportunity for their existence. So far 39 herbivorous arthropod species including two mites and three pollinators were found on the weed around Tsukuba city. The herbivore community was rather simple in terms of species diversity and feeding guilds being dominated by a leaf chewing coccinellid beetle *Epilachna vigintioctopunctata* (Fabricius). The beetle occurred two generations in a year on the weed and both of the larvae and adults caused a serious damage on the leaves. The feeding damage reduced flower and fruit settings of the weed while the number of adult beetles recruited depended on the foliage abundance of the weed in the field.

10-125

SECONDARY USE OF GALL MADE BY APHID ON MOUNTAIN ASH

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Gall making aphids, *Sorbaphis chaetosiphon*, damage directly to a mountain ash *Sorbus commixta* in May, which enhances leaf abscission early in the season. Plant structure will provide a shelter for associated insects. In this context, we focus on aphid galls as shelters for secondary users on a mountain ash. The gall is colonized throughout the season by secondary using insects, not only herbivores but also predators. The secondary using herbivore should take advantage from gall structure as a shelter. It might increase damage by herbivore. On the other hand, the occupation of gall by predators might result in negative enemy-mediated interaction between gall-making aphid and the secondary using herbivore. It might decrease damage by herbivore. Therefore, the gall making aphids would play an important role in altering interactions among insects on a mountain ash.

10-127

HOW CABBAGE DENSITY AFFECTS THE ARTHROPOD POPULATIONS ?

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Herbivorous and predatory species populations were examined in the fields with four different cabbage densities (0.25, 1, 4, 8 plants per square meter). The number of eggs per plant significantly decreased with the increase of plant density for three lepidopterous cabbage pests: the small white butterfly *Pieris rapae crucivora* Boisduval, the diamond-back moth *Plutella xylostella* (Linnaeus), and the beet semi-looper *Autographa nigrisigna* (Walker). The number of alate adults of the green peach aphid *Myzus persicae* (Sulzer) per plant showed a similar relationship. The population of spiders and that of syrphid flies per plant also decreased with the increase of plant density. On the other hand, the population per unit ground area increased approaching a maximum value with the increase of plant density for all arthropod populations. In order to clarify the cause of such a decelerating increase in the arthropod populations, the oviposition behavior of female *P. rapae crucivora* was observed. The oviposition process was divided into two components: the frequency of entrance of female adults into the field, and the frequency of oviposition from their entrance to exit. Both of the components contributed to the decelerating increase of the egg abundance per ground area and the latter component had a larger contribution.

10-128

EFFECT OF PREDATION BY THE CARABID BEETLE, *PARENA PERFORATA* BATES ON THE POPULATION CHANGE OF *THANATARCTIA IMPARILIS* BUTLER (COLEOPTERA: CARABIDAE – LEPIDOPTERA: ARCTIIDAE)

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In the southern area of Osaka Prefecture, southwestern part of Japan, the number of *Thanatarctia imparilis* colonies decreased abruptly from 1989 to 1991 and thereafter increased gradually from 1991 to 1995. The carabid beetle was observed in ca. 90% of the prey colonies in 1990, and it was not observed in 1991 and 1992. During 1993 to 1995, however, the density of *Parena perforata* increased as the density of *T. imparilis* increases. About 90% of *T. imparilis* larval colonies were destroyed before entering hibernation in 1990, however, the larval destruction was only ca. 10% in 1993 and 1994. In 1990, the carabid beetle deposited the eggs concentrating to the colonial nest, and many *T. imparilis* larvae of the nests were eaten by the carabid adults within a few days. In the laboratory, a carabid adult fed on ca. 100 *T. imparilis* larvae of the 1st and 2nd stadia per day. It is concluded that the predation by *P. perforata* acts as an important factor depressing the *T. imparilis* population.

10-130

SOME CHARACTERISTICS OF *CHRYSOPE CARNEA* (STEPH.) (NEUROPTERA: CHRYSOPIDAE) POPULATION COLLECTED IN THE SURROUNDINGS OF KISHINEV

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Peculiarities of photoperiodic response and feeding of insect adults, activity and molecular forms of their different enzymes have been studied during annual developmental cycle of green lacewing natural population. Polymorphism has been found in the studied population on the base of photoperiodic induction of the reproductive diapause. Typical changes in spectra of molecular forms of glucose -6- phosphate dehydrogenase and acid RNA-se have been revealed in insects entering diapause. They were shown to be associated with changes in food ration of insects giving their preference (during this season) to food rich in carbohydrates. Under natural conditions the diapause of lacewings was deepest in December and January. It was accompanied by maximum activity reduction of DNA-se, digestive alkaline phosphatase, certain molecular forms of glucose -6- phosphate dehydrogenase and malate dehydrogenase. Gradual recovery of activity and the number of molecular forms of studied enzymes was observed during reactivation period in Spring. The data obtained turned out to be rather useful for establishing rearing of *Chrysopa carnea* laboratory cultures capable of diapausing.

10-129

THE FAUNA AND TROPHIC RELATIONS OF THE FUNGIVOROUS-DWELLING BEETLES OF THE FAMILY CISIDAE (COLEOPTERA) IN WESTERN RUSSIAN PLAIN

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The family Cisidae is one of the most poorly studied coleopteran families in the East Europe. Cisid beetles are biologically connected with wood fungi.

Species composition and trophic relations of cisids were studied in the western part of Russian plain (western European Russia, Belarus') in 1989-1995.

In total, about 2000 individuals representing 31 species were collected. Records of some rare and relict species *Xylographus bostrychoides* (Dufour), *Ennearthron palmi* Lohse, *Orthocis lucasi* Ab., *Cis bidentulus* Ol., *C. fissicornis* Mellie, *Wagaicis wagaie* (Wank.) are noteworthy.

Twenty one wood-rotting basidiomycete fungi were recorded as host species for cisid beetles. *C. boleti* (Scop.) and *S. affinis* (Gyll.) appeared to have the widest host-ranges. Fungi species, condition and humidity of fruiting bodies are the main factor affecting distribution and occurrence of cisid beetles.

10-131

AN ECOLOGICAL CLASSIFICATION OF DROSOPHILID LARVAE (DIPTERA, DROSOPHILIDAE)

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Drosophilidae of the world fauna are divided into 4 trophic and 10 habitat groups according to larval nutritional substrate and habitat. The following trophic groups are proposed: 1) saprotrygophages, 2) mycetophages, 3) phytophages, 4) zoophages and cleptoparasites. The type of nutritional substrate corresponds with their habitats for larvae of mycetophages and phytophages, therefore, it is possible to consider them as mycetobionts and phytobionts. The habitats of zoophages and cleptoparasites are determined by various habitats of their prey or hosts, and we assigned these drosophilids to the group of heterohabitat species. The large majority of drosophilid larvae feed on different microorganisms of decaying substrates, especially on the yeasts, therefore, we name them saprotrygophages. The habitats of these species are very diverse, and 7 groups are proposed: phytosaprobionts, xylosaprobionts, mycetosaprobionts, zoosaprobionts, coprobionts, heterosaprobionts, commensals. Phytosaprobionts are divided into 3 subgroups: carpophilic, lachanophilic and anthophilic.

10-132

WHEN AQUATIC CHIRONOMIDAE (DIPTERA) FLY THROUGH LANDSCAPES

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In central Brittany (France), the adults' abundance of aquatic Chironomidae (Diptera) was studied by yellow trapping in four terrestrial habitats (the pondbanks, a woodlot, a mown grassland and a dry heathland) forming the transition area (ecotone) between a pond and terrestrial neighbouring systems. During one year, 43 aquatic species were found flying over these four sites, and their distribution was related to several habitat parameters: distance from the water collection, habitat openness, vegetation height and soil humidity.

The number of species as well as the total number of individuals were negatively correlated with the distance from the pond and with the habitat' closure, suggesting that aquatic chironomid species avoid densely vegetated and shaded areas which act as barriers while open and sunny terrestrial habitats are preferentially used as dispersal pathways.

The extent of the surrounding area influencing the species composition and abundance at each site was investigated using a Geographic Information System (GIS), allowing to compute Shannon's indices of habitats' diversity in a circle of progressively increasing radius around each trap.

A positive and significant rank correlation was only found between the species number at each sampling site and the habitats' diversity within a radius of 20 meters, suggesting that either flying abilities of the species are low or that adults perceive their environment at a small grain size (at a short distance).

The spatial organization and heterogeneity of landscapes is likely to influence the dispersal of adult Chironomidae originating in water collections. This fact seems of primary importance, both for the colonization of newly created or threatened aquatic habitats and for the permanency of aquatic communities, owing to the increasing fragmentation and modification of landscapes by human activities.

10-134

CARABID BEETLE COMMUNITIES OF CULTIVATED AND UNCULTIVATED AREAS IN NORTH-EASTERN ITALY (COLEOPTERA: CARABIDAE)

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Investigations were carried out in the lowland area near Pordenone (north-eastern Italy) to compare the populations of carabid beetles from three contiguous environments at increasing distances from a river: a riparian thicket, a natural hedge and a monocultural corn field. The soils, originating from fluvial sedimentation, were mainly sandy. Carabid communities were surveyed every two weeks from June 1991 to October 1992 by using pitfall traps containing an attractive-preservative solution (vinegar and 5% of formalin added).

A total of 5014 adults of Carabidae were trapped and 64 species identified.

The thicket community (794 adults belonging to 36 species) retained the characteristics of that of the original lowland woods and included forest species like *Cychrus caraboides* (L.) and *Abax carinatus* (Duftschmid).

The hedge community (584 adults belonging to 42 species) included many species from both the adjacent habitats.

In the corn field (3636 adults belonging to 40 species) mainly eurytopic species were found; *Pseudophonus griseus* (Panzer) and *Pseudophonus rufipes* (Degger) were largely dominant in the samples; the number of species was comparable with that of the other environments, whereas the number of individuals was much larger.

Riparian carabids, including *Elaphrus* F., *Asaphidion* Gozis and *Ocydromus* Clairville species, were present in all the sites.

The density of activity in the thicket reached its maximum between mid spring and mid summer; in the hedge and in the corn field it occurred later, between mid summer and the beginning of the autumn.

10-133

PERCEPTION OF LANDSCAPE STRUCTURE BY EMPIDIDAE (DIPTERA): WHICH SCALE?

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To study relationships between landscape structure and diversity and distribution of Empididae, adults have been sampled in three different areas using colored water traps. These landscapes are characterized by agricultural mosaic in which fields are surrounded by hedgerows. The Empididae scale of landscape perception is not a priori known. To determine the relevant scale at which the landscape must be described, we varied the extension of the study area around each sampling point. We started from a local scale at the hedgerow level, and extended step by step a window centered on this point (0.25, 1, 4, 16 and 64 hectares).

The most relevant scale happens to be an area of 4 hectares, which allows us to consider Empididae as fine grain species. The landscape mosaic is not the only factor accounting for species and community organization and one has to consider rather the interactions with the hedgerow structure.

If all Empididae are fine grain species they do not react the same way to the landscape. Functional groups of species may be defined according to the relevant scale of perception and to their relationship with landscape structure. These groups are characterized by the biological traits of their species. For example, some species of *Hilara* genus have to fly from emergence sites to confined mating sites. This leads them to use a greater space than comparably less mobile species.

10-135

DECAY RATES FOR SLUG ESTERASES WITHIN CARABID PREDATORS EVALUATED BY USING ISOELECTRIC FOCUSING

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In order to determine slug predation by carabid beetles in field, the rate at which prey esterases decay within a predator during digestion must be calibrated.

Carabid beetles (*Carabus cancellatus* Ill., *C. granulatus* L. and *Pterostichus melanarius* Ill.) which had been shown to feed on the pest slug *Arion lusitanicus* Mab., were kept in laboratory and fed with tissue of digestive gland of *A. lusitanicus*. The effects of consumption rate, digestion period and temperature were measured. Results will be presented.

10-136

ADULT POPULATION DYNAMICS OF TYPHLOCYBINAЕ, PENTHIMIINAE AND DELTOCEPHALINAE IN VINEYARDS OF NORTH-WESTERN ITALY (HOMOPTERA CICADELLIDAE)

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More than 30 species of Cicadellidae were captured in two vineyards of the Piemonte region using yellow sticky traps throughout all the growing season. The population dynamics of the most common species, belonging to the subfamilies Typhlocybinae, Penthiminae and Deltocephalinae, has been checked.

The most abundant species were *Empoasca vitis* (Goethe), *Zygina rhamni* Ferrari and *Scaphoideus titanus* Ball, all typically associated with grapevine. Many other species, living on vineyard weeds or on shrubs and trees growing close to the vineyards, were captured in a high number too. Some of these species, i.e. *Penthimia nigra* (Goeze) and *Anoplotettix fuscovenosus* (Ferrari) have important relationships with grapevine since they feed and lay eggs on it.

Two population peaks were shown by *E. vitis* at the end of June and at the beginning of August, and by *Z. rhamni* at the end of July and in October. *P. nigra* was trapped in May and June, *A. fuscovenosus* in June-August, *Fieberiella florii* (Stål) in August-September, *Macrosteles sexnotatus* (Fallén) in May and October. *S. titanus* adults were present from July to September, showing a peak at the beginning of August. *Thamnotettix* spp. were trapped in April-June while *Circulifer fenestratus* (Herrich-Schäffer), *Euscelidius variegatus* (Kirschbaum), *Psammotettix* spp. were caught all along the growing season.

The sex ratio of the above species was checked. For some species, namely *C. fenestratus* and *S. titanus*, yellow sticky traps proved to attract more males than females.

10-137

TEMPORAL CHANGES IN GUILD STRUCTURE OF PARASITIDS OF THE PEACH LEAF MINER (*LYONETIA CLERKELLA*) (LEPIDOPTERA: LYONETIIDAE) IN PEACH ORCHARDS WITH DIFFERENT CONTROL INTENSITY.

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The parasitoid complex of the peach leaf miner, *Lyonetia clerkella*, was investigated in peach orchards in Tsukuba, Japan, where the different numbers of spraying insecticides, i.e. different control intensity, were performed. Seasonal changes of the host density were estimated by the stratified sampling method, indicating that the host species has 6-7 generations in a year with each generation scarcely overlapping. In the non-insecticide-spraying orchards the host density which was high in early generations decreased with the generation, whereas the reverse curve of the host density was drawn in the insecticide-spraying orchards. *L. clerkella* larvae were sampled and the parasitism rates were estimated. Seasonal changes of the guild structure and the species diversity of the parasitoids were also examined. Nineteen species of parasitoids were identified from *L. clerkella* larvae and pre-pupae of the peach leaf miner: *Chrysocharis nitetis*, *Chrysocharis pentheus*, *Chrysonotomyia lyonetiae*, *Chrysonotomyia* sp. A, *Chrysonotomyia* sp. B, *Cirrospilus* sp., *Closteroceras trifasciatus*, *Desmatocharis* sp., *Minotetrastichus* sp., *Neochrysocharis* sp., *Pleurotropopsis japonicas*, *Stenomiesius japonicus*, *Pnigalio* sp., *Quadrastichus* sp., *Teleopterius delucchii*, *Teleopterius erxias*, *Zagrammosoma isonoi*, *Trichomalopsis* sp., and *Pholetesor* sp.

10-138

EFFECTS OF UVB AND OZONE ON INDUCTION AND INSECT FEEDING

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Very little is known about how anthropogenically altered ozone (O₃) levels and increased UVB radiation to the Earth affect plant phytochemistry. Phytochemical induction resulting from ozone and UVB damage are compared to natural herbivore damage. Changes in plant resistance to specialist insects are discussed for three insect-plant systems: cucumber beetle-squash, mexican bean beetle-soybean, cottonwood leaf beetle-cottonwood. Comparison of damage from herbivores and global change (ozone and UVB) revealed similar responses in all three insect-plant systems. In all three systems, induction resulted in greater specialist insect feeding. Flavonoids are increased as a result of UVB and defensive systems as a result of herbivory. In some plants, one system took over the function of the other, if one system was absent.

Studied were two species of squash (garden squash, *Cucurbita pepo*; and an endangered native, *C. okeechobeensis*), and three types of watermelon (domestic American, *Citrullus lanatus vulgaris*; naturalized Australian, *C. lanatus*; and native African, *C. colocynthis*). Total cucurbitacins increased (induction) for most species in most treatments with higher levels of cucurbitacin D associated with decreases in cucurbitacin I. Insects prefer these leaves. Production of cucurbitacin glycosides (E and I) are associated with the highest damaged (UVB and ozone) in all species. Cucurbits produce few flavonoids. In soybeans, a flavonoid kemperol increased as a result of any damage, and insects prefer these leaves. In cottonwood, the phenol glycoside salicin increased as a result of herbivory, and insect feeding increased. A flavonoid pinocembrum increased from UVB damage.

10-139

THE MOST SIGNIFICANT ENVIRONMENTAL FACTORS INFLUENCING THE NUMBER, DISTRIBUTION AND SEASONAL ACTIVITY OF THE WEEVILS (COLEOPTERA: CURCULIONOIDEA: APIONIDAE, CURCULIONIDAE) IN THE TATRA MTS.

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In the Tatra Mountains altitudinal effect is the most significant factor affecting the number, distribution and time of appearance of imaginal weevil forms which inhabit the above-ground parts of plants. With the altitude the weevil populations change. Both the number of species and the number of specimens drop. There are fewer lowland and more mountain species, the period of maximum occurrence is delayed and the time of occurrence shortened.

The major meteorological factors influencing the character of weevil occurrence are: 1) the time a compact snow cover lingers on the ground; 2) air temperature and, indirectly, 3) the Carpathian fen (halny) wind.

The species composition of the Tatra Mts. weevils depends, to a large extent, on the phytosociological structure of plant biotopes which constitute a feeding base for these beetles. The degree of vegetation density, especially of trees and shrubs in the forests affects their phenology too.

The host plant species play the secondary role in the distribution of mountain weevil beetles in the function of altitude.

10-140

POPULATION STRUCTURE AND REPRODUCTIVE OUTPUT IN A PALEARCTIC GROUND BEETLE, *ANISODACTYLUS SIGNATUS*
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The seasonal activity, age structure and reproduction of *Anisodactylus signatus* were studied on beetles collected by pitfall trapping in two regions of Hungary. Adult beetles showed two seasonal activity peaks: during mid-June, only "old" (overwintered) individuals formed a smaller activity peak, coinciding with the peak of the egg production. The second activity peak was in mid-July, when more than 86% of the collected individuals were "young". Egg numbers in the ovaries were low (2-4 eggs/gravid female). Females probably do not reproduce during their first adult year; in the subsequent season, they lay an estimated total of 15.56 eggs. The occurrence of iteroparity is not proven. *A. signatus* has a mixed overwintering strategy, and the age structure of the populations demonstrated regional differences. In eastern Hungary, no adults remained in the surface-active population after mid-summer; in central Hungary, adult activity continued into late September. The estimated total number of eggs significantly differed between the two regions. The significance of regional differences in population structure and reproduction is discussed.

10-142

POPULATION DYNAMICS OF SIMULIDAE (DIPTERA) IN THE MIDDLE VALLEY OF THE NEGRO RIVER (ARGENTINA).
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The middle valley of the Negro river (Río Negro province) in the North of Patagonia is an agricultural and cattle breeding temperate region. During the last decade the black flies became one of the most annoying pest. Farmers also claim for economic losses on cattle and some recreational areas are heavily affected in the summer season. The study was conducted to reach a better understanding on the population dynamic of the local species. *Simulium* (*Psaroniocompsa*) *bonaerense* Coscarón and Wygodzinsky, *S. (Ectemnaspis) wolffhuegeli* (Enderlein) and *S. (Pternaspatha) bachmanni* Wygodzinsky and Coscarón were observed. Sampling of natural and artificial substrate in representative habitats (Negro river, Salado river, irrigation channels and drainage channels) was carried out during the last three years (1993-1995). The three species are present the whole year with alternation of the predomination of them. *S. bonaerense* and *S. wolffhuegeli* were found breeding in all water courses predominating in habitats with wide range of conductivity (50-558 mS), while *S. bachmanni* was only found breeding in the Negro river (conductivity: 90-192 mS). *S. bonaerense* predominates in all water courses but the river, where *S. bachmanni* and *S. wolffhuegeli* predominates alternatively according to a seasonal pattern. It was observed that *S. bonaerense* is the most aggressive and anthropilic species within the area.

10-141

PATTERNS OF ATTACK ON BAIT STAKES BY *MASTOTERMES DARWINIENSIS* FROGGATT AND SYMPATRIC SPECIES IN TROPICAL AUSTRALIA (ISOPTERA: MASTOTERMITIDAE)
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Grids of timber bait stakes have long been used to monitor termite activity and foraging patterns. Data from the first year of a long-term study of the foraging patterns of *Mastotermes darwiniensis* Froggatt are presented. The results to date indicate that in the Darwin region, where 6-8 species compete for baits consisting of unweathered radiata pine stakes, inter- and intra-specific colony boundaries are highly dynamic. Very few stakes inspected at six-weekly intervals were continuously attacked by the same species and some have never been attacked by any species after one year. Although figures are incomplete in that a full wet-season dry-season cycle has yet to be completed, there is little seasonal variation in *Mastotermes* activity apart from a predictable reduction following alate swarming.

10-143

AUSTRALIAN FRUIT FLY DISTRIBUTIONS

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The distribution of fruit flies in endemic regions of Australia has been monitored for three successive years during February/March through National Fruit Fly Experiments. The samples were collected in collaboration with the CSIRO's Double Helix Club members. In the first year, 2,000 volunteers were provided with a lure/insecticide bait and instructions for constructing a trap. In subsequent years, sampling was repeated using a more limited number of volunteers to cover the range. Flies were classified and stored for DNA analysis. The classifications indicate trends in distributions of Australian fruit fly species.

10-144

CARABID COMMUNITIES FROM CORYNEPHORETA AND CALLUNA HEATHS IN NORTHWEST-GERMANY: COMMUNITY STRUCTURE, DISTRIBUTION, AND DISPERSAL (COLEOPTERA: CARABIDAE)

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The landscape in Northwest-Germany is man-made and was modified several times. Actually, only a few remnants of Coryneporeta on drifting sands and Calluna heaths are left from a former countryside dominated by them until the 18th century, when deforestation reached its maximum.

In 1995 the composition of the carabid fauna from the above-mentioned plant communities was investigated by pitfall trapping on six sites in Northwest-Germany. These sampling sites differ in total area and all are completely isolated from each other by surrounding forests or agricultural land.

Several stenotopic species of Coryneporetum-communities or open sparsely vegetated parts in dry heathlands were sampled, but the complete spectrum of potentially occurring stenotopic species could not be found on any site. At least one species, *Cicindela sylvatica*, is supposed to have totally disappeared from sandy and heathy localities in the studied area in the course of the last decades.

The number of stenotopic species decreases as expected in small and fragmented habitats. Four brachypterous species with low dispersal power were caught, each of them in only one or two of the sampling sites. Since their populations must have survived the last decades in restricted areas, they are highly endangered by extinction, especially if the habitat quality of these small patches gets less suitable for them. In contrast, characteristic winged species of these habitats are widely distributed in the investigated area, indicating that dispersal power is an important factor for survival in a changing landscape with small and isolated habitat islands.

10-145

COMMUNITY EFFECTS OF THE INTRODUCED GROUND-BEETLE *PTEROSTICHUS MELANARIUS* (ILL.) IN WESTERN CANADA

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The distribution and community effects of the ground beetle *Pterostichus melanarius*, of European origin, were studied in western Canada. Pitfall trapping showed that the species is one of the most abundant carabids in the city of Edmonton, and is also common up to ca. 70 km from the city. The proportion of macropterous individuals of this dimorphic species increased from ca. 20% in Edmonton to 60-70% in the most distant populations. Contrary to previous studies in anthropogenic habitats, *P. melanarius* did not show negative pairwise associations with any of the abundant native species.

We also used a four-year field experiment to examine potential adverse effects of this species on a native carabid assemblage in an aspen-poplar forest. Three enclosures were each divided into six 4 × 4 m compartments, and the following treatments (with two replicates in each enclosure) were used: (1) *P. melanarius* alone, (2) six abundant native species alone, and (3) *P. melanarius* together with the six native species. *P. melanarius* maintained breeding populations in all compartments where introduced, but it had no negative effect on population size or body mass of the native species. However, the two most abundant native species (*Pterostichus adstrictus* and *P. pensylvanicus*) appeared to be more active in compartments where they co-occurred with *P. melanarius*. Although the enclosures were just a few meters apart, a strong enclosure effect was detected with one of the three enclosures yielding the highest numbers of both the native species and of *P. melanarius*. This difference was correlated with high abundance of earthworms and low litter accumulation in that enclosure. Low densities of *P. melanarius* in forests may explain the lack of negative interspecific interactions in our experiments, though these are suggested in data from urban and agricultural environments where *P. melanarius* is more abundant.

10-146

EVIDENCE FOR INTERPLANT COMMUNICATION OF WOODY PLANTS IN THE FIELD - EFFECTS ON THE HERBIVORE GUILD OF *ALNUS GLUTINOSA*

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Consequences of manual defoliation on the herbivore guild of alders (*Alnus glutinosa*) were studied in the field. One tree in each of 10 alder stands was damaged in spring by manually removing approximately 20% of total leaf area. After resprout, subsequent leaf damage by alder leaf beetle (*Agelastica alni*) and abundance of other herbivores was assessed on 10 trees differing in distance to the defoliated alder until the end of the vegetation period. Stem diameter and infestation with gall mites proved to be minor factors influencing leaf damage caused by the beetles. Effects of manual defoliation explained leaf damage best, which significantly increased with distance to the defoliated tree. Abundance of phytophagous insects rose likewise. Accordingly, induced resistance caused by manual defoliation not only reduced herbivore performance on the defoliated tree itself, but also on its neighbours. These results show strong evidence for interplant communication in *A. glutinosa*, and could be supported by laboratory tests.

10-147

AN ANALYSIS OF POPULATION SYSTEM SPATIAL STRUCTURE OF THE BEET WEBWORM *LOXOSTEGE STICTICALIS* L.

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In this paper a local population of *L. sticticalis* survival at the variation of photo - thermal conditions are studied using the space of concordance of inner (physiological) and external (astronomic) times, where inner time is determined as a sum of efficient temperatures. For the beet webworm the sum of efficient temperatures of development stages and their combination are thermal constants. The inner time of a sensitive phase -- the third, fourth, and fifth age of larvae stage is also constant. We fix its respective values on the axis of inner time.

On the axis of external time a value of photo threshold is considered, the value is determined by only the geographic latitude of a local population. The population is described by a set of temporal stratum in external time. Thus, if the sensitive phase of certain stratum develops after threshold and has a necessary sum of efficient temperatures for the survival in accordance it the LCj - type life cycle than the percentage of diapausing individuals will be greater than zero.

This paper shows that at the absence of migrations in the system the trajectory variety of temporal stratum in the space of concordance of inner and external can be divided only into four types. Respectively, the population system range consists of four types: survival region of LCj - type life cycle, "overheating" region, degradation region, and region of disharmony. A detailed spatial structure of British - Ob population system also is described here.

10-148

DRIFT OF RESPONSE OF THE BEET WEBWORM
LOXOSTEGE STICTICALIS L. (LEPIDOPTERA, PYRALIDAE) ON
VARIATION OF ITS POPULATION DENSITY

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At present abundance dynamics of *L. sticticalis* is considered only as a passive reaction to a change of modifying factors. However, the result of our recent research have shown that there is non - trivial modulation of external effects by means of a set of endogenous transformations having certain inertia. The inertia is disclosed itself as a dependence of internal population state on its previous history.

Effect of density on the abundance dynamics of the beet webworm at various stages of gradation cycle has been studied. Comparison of results obtained in 1991 and 1994 explicitly shows the drift of the following parameters: mortality, structure of larvae color morphs, weight structure of population and female fecundity. Direct influence of previous history on the structure larvae color morphs also has been studied. It has appeared that the morph structure determined as by current density as population density of the preceding generation.

Thus, the obtained results allow to conclude that abundance dynamics of species is determined not only by environmental parameter changes but also by dynamics of endogenous parameters.

10-150

EFFECT OF DENSITY ON PHOTOPERIODIC REACTION OF BEET
WEBWORM *LOXOSTEGE STICTICALIS* L. (LEPIDOPTERA:
PYRALIDAE)

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The density is one of the main population characteristics. Its change causes a cascade of transformations of the population structures. However, there are comparatively few records about effects of population density on diapause and photoperiodic reaction (PhPR) of insects. Such data for beet webworm are absent. The study was conducted on a first generation larvae. Imago were collected on the south of Novosibirsk region (South of Western Siberia). In each variant of photoperiod (12, 13, 14.5, 16 hours) were 3 variants of density - 5, 10 and 50 larvae per glass container (volume 0.5 l). Larvae have finished development on August 3-5. After 45 days (on September 15) the account of diapaused pronymphs was conducted. Larvae developed at $t=26 \pm 1^{\circ}\text{C}$. PhPR of beet webworm was in detail studied by Saulich et al.(Zool. Zh., 1983,) on populations from European part of Russia. Our data (Tabl.1) suggested that the increase of population density causes significant decrease in percent of diapausing pronymphs. Low percent of diapausing pronymphs is marked at shortday photoperiod (12 and 13 hours). Following data of Saulich et al. beet webworm is a species with a thermostable PhPR of long day type and without geographical variability of its parameters. The contradiction between our data and results of Saulich's et al. studies gives evidence of insufficient studying of a beet webworm PhPR. We have found out the drift of such important parameters as fecundity and structure of color morphs (unpublished data), that allows to speak about population cycle of beet webworm. Quite probably, that the character of PhPR can differ in different phases of population cycle.

Tabl.1. Effect of density on PhPR of beet webworm. Numbers are percentage of diapausing pronymphs $\pm Cl_{0.95}$ (confidence interval)

Photoperiod Density	12 hs	13 hs	14.5 hs	16hs
5 larvae/0.5l	53.8 \pm 13.1	23.0 \pm 11.0	67.0 \pm 21.2	0
10	58.0 \pm 15.8	20.0 \pm 11.9	55.0 \pm 18.7	0
50	13.0 \pm 4.0	4.0 \pm 3.6	16.0 \pm 8.6	0

10-149

A STUDY OF MIGRATIONS OF THE BEET WEBWORM
LOXOSTEGE STICTICALIS L.: A QUANTITATIVE APPROACH
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It is well known that the migrations result in a change of spatial structure of the population systems. The region of degradation are reduced for account of the arising of migrant survival region. The effect is explained by that the migrant inner (physiological) time is compound and determined by photo - thermal conditions of the respective compartment set. At availability of migrations in the system the trajectory variety of temporal stratus in the space of concordance of external (astronomic) and inner times can be divided into five types. Respectively, population system range consists of five types: survival region of LCj - type life cycle, region of migrant survival, "overheating" region, degradation region, and region of disharmony of inner and external times. The first and second regions can be sources of migrations if the local population density execs threshold value. Migrational induction in the system results in a significant expansion of the effective range territory where processes of reproduction and dispersal occur. As a result outbreak of *L. sticticalis* appear.

Thus, using space of concordance of inner and external times permits: 1) to define a detailed spatial structure of population system at each stage of gradation cycle, 2)to explain a phenomenon of extension of effective territory of the range by migrations, 3) to calculate ecological consequences of all possible migrations in the population system of *L. sticticalis*.

10-151

THE TRANSPLANTING AFFECTS THE SPATIAL
EXPANSION OF A NEWLY COLONIZED INSECT, RICE
WATER WEEVIL, *LISSORHOPTRUS ORYZOPHILUS*
(COLEOPTERA, CURCULIONIDAE) IN JAPAN.

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The Rice water weevil (RWW) *Lissorhoptrus oryzophilus*, which is likely to have invaded from California, is currently one of the major pests of rice in Japan. In most of Japan, RWW has one generation a year, and overwinters as adult in grass leaf litter near paddy fields.

In spring, adults feed on grass near the overwintering site, and the flight muscle develops. After they disperse to paddy fields, where they feed on the leaves of rice and begin to oviposit.

RWW was newly found in Nagoya, the central part of Japan in 1976. It expanded its distribution across prefectures and distributed all over Japan in 1986. While they were expanding the spatial expansion rate in each prefecture was greatly different. The ratio was negatively correlated with the sum of effective temperature till transplanting date of rice in each prefecture.

The oviposition of RWW depends on the transplanting of rice, because the development of the ovary of the adults needs feeding of the rice leaf and eggs must be oviposited in the stems of rice transplanted in a paddy field. The development of flight muscle, the development of ovary and the process of oviposition depend on the sum of effective temperature.

In the aria where the sum of effective temperature till transplanting date of rice is large, transplanting is later than when the sum of effect temperature enough for ovary development is attained. As the result, RWW can not feed for the development of ovary which results in reducing the population growth. This may be the reason for the smaller expansion rate in the preference where the effective temperature is large and vice versa.

10-152

PREDACIOUS MITES (ACARI: MACROCHELIDAE) AND SYNANTHROPIC FLIES (DIPTERA: MUSCIDAE AND FANNIDAE) ATTRACTED TO ACCUMULATED POULTRY MANURE FROM FARMS OF ARGENTINA.

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Many species of synanthropic flies and mites predators of fly eggs and first instar larvae are the most frequent organisms in the manure from poultry farms using caged-layers.

The present work was undertaken to know the variety of flies (Muscidae and Fanniidae) and mites predators associated to poultry manure accumulations in production systems from the South East of Argentina.

During 1994, 1995, samples of accumulated manure were collected at monthly intervals, in barns from three farms from the South-East of Buenos Aires province, Argentina.

Mites of the genus *Macrocheles*, *Fuscouropoda*, *Parasitus*, *Eugamasus*, *Poecilochirus*, *Hypoaspis*, *Proctolaelaps*, *Arctoseius*, *Sejus*, and fly-larvae of the genus *Musca*, *Fannia*, *Ophyra* and *Muscina* were extracted from the manure by illuminated tullgren funnels and identified. Although twelve mite species not previously recorded to the site were counted, *Macrocheles muscaedomesticae* was predominant followed by *Fuscouropoda* sp. Fly larvae of *Musca domestica*, *Fannia canicularis*, *Ophyra aenescens* and *Muscina stabulans* were encountered in that order of abundance. The population numbers of the predators *M. muscaedomesticae* and *Fuscouropoda* sp. coincided seasonally with *M. domestica*, *O. aenescens* and *F. canicularis*.

10-154

POPULATION DYNAMICS OF SAN JOSE SCALE *Quadraspidiotus perniciosus* COMSTOCK, 1881 (HOMOPTERA: DIASPIDIDAE) IN PEACH ORCHARD, IN JACUÍ, MINAS GERAIS STATE - BRAZIL.

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The population dynamics of San Jose Scale, *Quadraspidiotus perniciosus* on peach orchard in Jacuí, Minas Gerais - Brazil was studied. The natural enemies and weather effects were evaluated. The samples were made on branches of peach tree in two areas, being one treated and the other untreated from August/1994 to July/1995. Adult females occurred all over the year, and presented population peaks in August, October, January and March. Crawlers emerged early in August with increasing of the climatic factors. It showed three peaks in August, January and February. The natural enemies, principally, parasitoids and the fungus *Fusarium coccophillum* (Desm.) showed a high effect on *Q. perniciosus* in untreated area, with 9.5% of parasitized scales and 29.4% of infected scales. In treated area, 5% of parasitized scales and 15% infected scales resulted. The weather (temperature, relative humidity and precipitation) showed more effect on the pest in treated area. Temperature and precipitation had a greater influence on the stages of scales at 30 days before the samplings. In this study, *Q. perniciosus* presented higher population in treated area than in untreated area. The untreated area showed more equilibrium, that is to say, maintenance of *Q. perniciosus* population to lower levels because of the action of their natural enemies and also for climatic effects. The insecticides sprayed in treated area showed to be more harmful than the natural enemies.

10-153

LONG-TERM EFFECTS OF PEAT-CUTTING ON BEETLE (INSECTA: COLEOPTERA) COMMUNITIES OF A DAMAGED CENTRAL-EUROPEAN MOOR

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The coleopteran communities of the moor 'Rotes Moor' and the surrounding areas were investigated in 1993 and 1994. The study sites are situated in the Rhoeun-mountains (Germany), about 810m above sea level. The peat-bog has been damaged by drainage and commercial peat-cutting until 1984. Northern-palaeartic-boreal and Central-European-mountainous species dominated the coleopteran communities. Typical associations between various beetle species and characteristic vegetation types (moor, cut-over area, swamp, surrounding *Betula-carpatica*-wood and *Picea abies*-forest) could be established.

It is shown that the long-lasting alteration of vegetational patterns towards heath following drainage and peat-cutting is the most striking factor, causing a dramatic change of the original beetle community. Tyrphobiontic species were nevertheless found in small relicts of growing moor-areas. The wet cut-over areas, in contrast, were colonized by beetle-communities similar to those of the swamps surrounding the moor in which tyrphobiontic species have completely vanished. The results are discussed in the context of the effect of peat-cutting and drainage on niche-choice behavior of some abundant species. In addition, it is demonstrated that differences in phenological patterns lead to niche separation between closely related, syntopic species of Staphylinidae (*Liogluta* spp.).

10-155

POPULATIONAL DYNAMICS OF THE PEACH APHID *Brachycaudus* (*Appelia*) *schwartzi* (HOMOPTERA: APHIDIDAE) IN JACUI-MINAS GERAIS STATE, BRAZIL.

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One of the factors which contributes to low yield of the peach tree in Minas Gerais, state-Brazil, is the occurrence of *Brachycaudus* (*Appelia*) *schwartzi*, which causes leaf and shoot rolling and leads to side sproutings, harming the plant architecture.

This research had the objective of studying the population dynamics of the peach aphid *Brachycaudus* (*Appelia*) *schwartzi*. The weather effects on this aphid population were evaluated, as well as the distribution of first to fourth instar nymphs both apterous and alate and also apterous and winged adults.

Three apical branches approximately 25 cm long per plant on 10 plants giving preference to branches which had shown greatest aphid colonies, were sampled fortnightly, from August, 1994 to July, 1995. All over the survey period, the presence of *Brachycaudus* (*Appelia*) *schwartzi* was found, where its population proved to be positively correlated with temperature and negatively with relative humidity. The highest populational peak was achieved on September, 1994, where the temperature and relative humidity accumulated seven days before the samples were 24.3 °C and 55.8% respectively.

The occurrence of first and second instar nymphs was greatest relative to the other stages, standing for 62.68% of the total of sampled individuals. The occurrence of apterous third and fourth instar nymphs was 17.07%, value which is superior to that found for alate third and fourth instar nymphs which was 2.78%. The percentages of apterous and winged adults were 16.64% and 0.83%, respectively.

10-156

INVASION ECOLOGY OF *SOPHONIA RUFOFASCIA* (HOMOPTERA: CICADELLIDAE), THE HAWAIIAN GOURMAND

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The two-spotted leafhopper, *Sophonia rufofascia*, is a recently introduced insect that feeds on over 300 species of plants and is changing the landscape of Hawaiian forests and watersheds. Particularly hard-hit plants include the false staghorn fern (uluhe), Hawaiian tree fern (hapuu), *Metrosideros polymorpha* (ohia lehua), and *Myrica faya* (firetree). Studies are underway to better understand plant response to *Sophonia* feeding; patterns of uluhe dieback and dead patch revegetation; *Sophonia* ecology relative to patterns of plant host attack, and the diversity and impact of natural enemies (principally egg parasitoids); and the possibility that *Sophonia* may threaten the health of rare endangered Hawaiian plants.

10-158

ANALYSIS OF ORIBATID MITES (ACARI: ORIBATIDA) COMMUNITIES IN MT. JUMBONG IN KOREA

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Analysis of oribatid mites communities was conducted at 4 sites (the plantation of Korean pine in alt. 900m; the mixed forest in alt. 1100m; the north-facing slope where lower vegetations were annual herbaceous, and the south-facing slope where lower vegetation was perennial sasa in alt. 1000m, and these slopes were adjacent to each other) in Mt. Jumbong which is a nature reserve area in Korea. Also, organization of oribatid mites communities in the decomposition process of litter was investigated at north- and south-facing slope. The study was conducted from June 1994 to Oct. 1995. In total, 52 families, 81 genus, and 143 species of oribatid mites were collected. Species composition, densities, and dominant species were significantly different between north- and south-facing slopes. About 70% of total species and density were found in less than 5cm depth of soil. *O. nova* was common dominant species at 4 sampling sites. *P. duplicata nipponica* was a dominant species at south-facing slope but was not found at north-facing slope. *Boreozetes donghaksensis* was a dominant species at mixed forest in alt. 1100m, but a rare species at other sites. Species diversity (H') was higher at south-facing slope ($H' = 3.06$) than at north-facing slope ($H' = 2.67$). The proportion of influent species was very low at alt. 900m site compared to other sites. The values of similarity index among sites or sampling dates were higher than 0.6. Results of MGP analysis indicated that M-type and G-type species were dominant groups in Mt. Jumbong.

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NATURAL HISTORY OF A GALL-FORMING WEEVIL, *COLLABISMUS CLITELLAE* (CURCULIONIDAE), ON *SOLANUM LYCOCARPUM* (SOLANACEAE) IN SOUTHEASTERN BRAZIL.

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Collabismus clitellae promotes gall formation in branches of *Solanum lycocarpum* (Solanaceae), an invading species in areas of economic value with antropic influence. *C. clitellae* individuals are distributed in variable-sized galls (which are determined by the number of chambers present in each gall), occurring preferentially on the basal part of smaller-sized plants. The adult weevils have a great variation in size (range 4 - 10mm). In this study we relate the main causes of mortality in individuals of *C. clitellae* and describe the development stages and weight variation of the pupes in relation to the gall characteristics and the distribution in the host plants. We collected 376 galls randomly in a population of *S. lycocarpum*, located at Serra do Cipó in southeastern Brazil.

The number of eggs laid by *C. clitellae* females increases with the branch diameter, decreasing with the host plant size. The gall volume increases with the number of chambers until a well defined limit. Therefore, very crowded galls are formed, leading to a decrease on the mean weight of pupae.

The *Penicillium* sp fungus and the woodpecker *Colaptes campestris* were the main external factors affecting the mortality of galls. The fungus occurrence was higher on shorter plants and bigger galls, but was independent of the position of gall on the host plant. Larger galls positioned on the higher parts of the host plants were more often attacked by woodpeckers. We suggest that the survival probabilities and the body size of *C. clitellae* individuals increases with the choice of oviposition sites and the clutch size. This choice is related to mechanical and biochemical differences between different-sized plants and by different mortality agents. Moreover, the fungus and bird attacks must be important factors regulating the population size of this gall species.

10-159

SEQUENTIAL SEED PREDATION ON *PITHECELLOBIUM TORTUM* MART. (LEGUMINOSAE)

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In the Restinga de Barra de Maricá, Maricá, Rio de Janeiro, Brasil, *Pithecellobium tortum* immature seeds are attacked by larvae of *Allorhogas dispistus* (Braconidae) and maturing seeds by larvae of *Merobruchus boucheri* (Bruchidae). In order to investigate whether predation by braconids affects subsequent resource utilization by bruchids we collected 400 fruits from 8 trees in 1992 and 291 pods from 10 trees in 1995. All pods and seeds were dissected and insects or their damage recorded to ascertain predation rates from different agents.

In 1992 *Allorhogas dispistus* was relatively scarce, attacking 31% of the examined pods (range 0 - 86% per tree) and 12% of the seeds (0 - 33%). In 1995, the braconid infested 76% (34 - 100%) of the pods and 54% (7 - 99%) of the seeds. Bruchid attack varied less between years: 71% of the pods and 20% of seeds were infested in 1992, 46% of the pods and 14% of the seeds in 1995. Among-tree comparisons indicated no effect of the braconid infestation on bruchids in 1992, but in 1995 both pod and seed infestation rates by the two predators were negatively correlated ($r = -0.82$ and $r = -0.88$ respectively). Nonetheless, though bruchid larvae were more numerous in trees less attacked by braconids, infestation rates were not higher on those trees. Results suggest that firstcomers affect later predators only above a fairly high density and, apart from reducing total resource availability, they do not provoke noticeable change in attack patterns among pods or trees.

10-160

THE EFFECT OF CADMIUM STRESS ON THE INTERACTIONS BETWEEN *PARARGE AEGERIA* AND ITS HOST PLANT.I. Vandevyvere¹, L. De Bruyn¹, R. Blust²

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Since anthropogenic pressure on ecosystems continues to increase, metal pollution will be one of the most important problems in the future. In the soil, heavy metals operate as stress factors causing physiological constraints after absorption by the root system. This often results in a decreased vigour of the plants and retardation of plant growth.

Insects are dominant components in many terrestrial food webs. They play an essential role as primary consumers of plants and in turn are an important source of food for the higher trophic levels. Consequently, the changes caused by pollution are not restricted to the plants themselves, but may also affect other parts of ecosystems. However, the behaviour and physiological impact of heavy metals on the different trophic levels of terrestrial food chains are poorly understood.

In this study, attention is focused on the impact of cadmium intoxication on *Holcus lanatus* and its herbivore *Pararge aegeria*. To assess cadmium concentration along the food chain more accurately we carried out laboratory studies. Of primary importance is the regulation of the levels of soluble and plant available Cd in soils. Therefore bioavailability was studied using soils with a range of Cd treatments. Dose-response experiments with moderate to excess metal supply to *Holcus lanatus* seedlings gave data on the direct impact of Cd on the plant fitness. We measured growth, biomass production, photosynthetic characteristics and the nutritive value as host plant. Grasses grown on soils with different cadmium treatments were offered to the herbivore larvae. Growth rate, final size and consumption efficiency were measured. The results are explained based on the nutritive host plant characteristics.

10-162

THE INFLUENCE OF PLANT STRESS ON HOST SELECTION AND PERFORMANCE OF THE GRASS MINER *CHROMATOMYIA MILII* (DIPTERA; AGROMYZIDAE)

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Environmental stresses such as water deficit, or nutrient imbalances, have been hypothesized to change plant quality in nature. Two major hypotheses were formulated. The plant stress hypothesis proposes that physiologically stressed plants become more susceptible to herbivores. On the contrary, the plant vigour hypothesis proposes that plants that are growing vigorously are favourable to herbivores. Recent research brought to the light that the result of plant insect relationships is largely dependent on the feeding behaviour of the herbivores studied. Endophytic insects (miners, gall-formers, borers) should prefer and do better on vigorous growing plants.

Environmental stress theories were largely based on insect guilds feeding on woody plants. Compared to more complex plants such as trees, grasses only possess simple plant architecture, relative low protein concentration, and a low diversity of secondary compounds. Therefore we can suppose the host plant will act differently on the herbivore. The aim of this study was to test the plant stress / plant vigour hypothesis for an internal feeding herbivore living on grasses. We selected for this purpose *Chromatomyia milii*, an oligophagous grass miner (Diptera; Agromyzidae).

In a greenhouse, under controlled conditions, we tested female host plant selection, larval mortality, larval growth rate and final adult size on grasses growing under different levels of nutrient and water stress.

Our results show that low egg densities and low larval performances occurred on grasses under low and high nutrient stresses. High egg densities and high larval performances occurred on grasses under medium nutrient stress. High egg densities and high larval performances occurred on grasses under high water treatment. Differences in food plant quality are responsible for the observed differences in female ovipositional preference and larval performance.

10-161

A COMPARISON OF DIRECT AND INDIRECT METHODS TO ESTIMATE THE POPULATION STRUCTURE IN A DAMSELFLY

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Traditionally population size and lifespan are estimated with indirect methods such as the Jolly-Seber (JS) method and the Manly-Parr (MP) method; lifespan with the method of Scott. An in odonatology scarcely used direct method to determine the population size is the Minimum Number Alive (MNA) method, developed by Krebs (1966) for capture-recapture studies of mice. A simple direct estimate of lifespan is the residence time in the population. We compare these methods, using field data on the damselfly *Lestes sponsa*.

The capture probability influences the accuracy of the population size estimates. The three methods give very similar estimates for the male population sizes. In females however capture probabilities are low. This reduces the number of days that MP and JS give accurate estimates. MNA underestimates absolute population size, but gives the opportunity to know the evolution in population numbers on all days. Estimates of lifespan by the method of Cook and directly by the residence time were the same for males. This last method, however, underestimates female lifespan due to the long interval length between two successive visits in females.

10-163

SURVIVAL AND NON-LINEAR TEMPERATURE-DEPENDENT DEVELOPMENT MODEL OF IMMATURE *AUBEONYMUS MARIAEFRANCISCAE* ROUDIER (COLEOPTERA: CURCULIONIDAE).

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The sugar beet weevil *Aubeonymus mariaefranciscae* Roudier is a new key pest of sugar beet in Southern Spain. Survival and development of immature stages were studied by rearing them, on artificial diet, at seven constant temperatures ranging from 15° to 33°C.

Eggs development occurred over the whole range of temperatures tested, though a large decrease in the hatching percentage was observed above 30°C.

First, second and third-instar larvae were able to develop at low temperatures but failed to do so above 30°C. Conversely, fourth-instar larvae did not develop below 18°C, and their developmental time at 20°C was longer than 50% of the total immature developmental time.

A non-linear function, Logan type III, rather than a linear one, provided a reliable fit of developmental rates (reciprocal of mean time for development) versus temperature for all immature stages, specially in the high and low-temperature range. The percentages of mortality vs. temperature are also showed.

Implications of these results in predicting *A. mariaefranciscae* phenology, under field conditions, are discussed.

10-164

PRELIMINARY STUDY OF ANT DIETARY-HABITS IN BORNEAN RAIN FOREST - Do ants play as primary consumers in tropical ecosystems ? -

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Ants are among the most abundant animals in tropical forests. Although the ant-predation should be considered to have a major impact on the composition of tropical invertebrate biodiversity, the dietary habits have not been studied for large majority of tropical ant-groups. In Sarawak, Borneo, we conducted food preference tests of 19 species (*Dolichoderus*, *Camponotus*, *Polyrhachis*, *Crematogaster*, *Leptogenys*, *Aenictus* ssp. etc.) and continuous observations of 5 species at trails near the nest to find captured prey.

Results

1. All species tested here accepted soil protein foods (cheese and dead insects).
2. *Aenictus* and *Leptogenys* ssp. accepted honey-solutions eagerly in food tests.
3. Continuous observation of *Dolichoderus cuspidatus senipennis* (Nomad ant) for 31hr. showed that only 2 workers carried dead arthropod (Total number of workers returning to the nest = 19,305).
4. In the observation of *Polyrhachis bihamata*, single worker carried dead arthropod (Total observation time = 4hr. Total number of workers returning to the nest = 1,488).
5. The observations of *Camponotus saundersi* and *Crematogaster inflata* showed that no workers carried soil protein foods to the nests.

10-166

POPULATION OF TERRESTRIAL CARABIDAE FROM DIFFERENT ZONES OF CENTRAL SIBERIA.

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The study of the Carabidae population in the natural ecosystems of Central Siberia was carried out in 1988-1995. The region of research is located on Asian transect (along Yenisei River) from the Sibiryakov Island in the north (73° N) up to the Predvinsk in the south (57° N) and covers eight subzones: arctic, typical and southern tundra, forest-tundra, northern, middle, southern taiga and subtaiga.

In our material were found out more than 100 species of the Carabidae family, large part from which concerns to the genus *Pterostichus*, *Carabus*, *Agonum*, *Bembidion*. The poorest communities of the ground-beetles is registered in arctic tundra (4 species). The most diverse ones was described in some southern subzones from the middle taiga up to the subtaiga (50-65 species).

In the ecosystems of arctic tundra only one species of the ground-beetle was dominant in most part of communities. For example, *Pterostichus brevicornis* was dominant in all sites on the Sibiryakov Island. 3-5 ground-beetle species inhabit each community in typical tundra and in the intrazonal coastal localities singly the widespread species were marked, for example, *Elaphrus riparius*, *Bembidion lunatum*. In the southern tundra there are some forest species in the *Alnus* association, for example *Trechus mortcowitschi*. In forest-tundra the complex of forest species is represented by many species, however concrete forest and tundra communities have a few similar species. Northern tundra species, for example *Pterostichus brevicornis*, penetrate rather far to the south up to the middle taiga, but in small quantity.

Boreal zonal ecosystems have rather a few number of species (5-10), in comparison with intrazonal communities of bogs and flood-plain, where the number of species is much more (25-30). In all kinds of a taiga in zonal interfluvial ecosystems only three species (*Pterostichus delutipes*, *P. ehnborgi*, *Calathus micropterus*) were basically dominant. In subtaiga zone to these three species is added *P. magus*.

10-165

DISTRIBUTION AND APPEARANCE OF THE SILVER Y MOTH, *AUTOGRAPHA GAMMA* (LEPIDOPTERA, NOCTUIDAE), IN JAPAN, AND SEASONAL CHANGE OF CHARACTERISTICS CONCERNING WITH MIGRATION

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The silver Y moth, *Autographa gamma* (L.), is a famous migrant insect pest in Europe. This species hibernate in Mediterranean area that has warm winter, and the moth migrate to north way in summer. Distribution of the silver Y moth in Japan recorded at first in 1960s and nowadays there are many records in all area of Japan. The species produce big population in Hokkaido Is. which has long, cold and snowy winter, although the species is few or rare in southern part, Honshu, Shikoku or Kyushu Is..

The moth appear 3 times, May-June, August, and September-October in Hokkaido. They have not obligatory diapause stage but they hibernate in middle larval stages. Dry weight of the male moth captured by pheromone trap in Memuro, Eastern Hokkaido, are light in order of first> second> third appearance. On the other hand fore wing length of the male moth elongate in order of first< second< third appearance. Hence, the weight-wing index (dry weight / fore wing length) become small in order of first> second> third appearance. The same results are taken in the data from four other places in Hokkaido. Dry weight and fore wing length of the female and the male moths captured by a light trap have the same results. Ratio of the mated female captured by a light trap are 90, 65 and 25% in the first, the second and the third appearance respectively. Seasonal changes of characteristics of the silver Y moth in Hokkaido indicate that the adults in third appearance physiologically and morphologically suit for migration.

10-167

INDIVIDUAL SEX PHEROMONE RESPONSE DIFFERENCES IN MALE *AGROTIS SEGETUM* MOTHS (LEPIDOPTERA: NOCTUIDAE)

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Several studies of insect mating systems have identified male traits, such as age and body size, that influence pairing success in the field. In moths is pheromone response also important. Here I show that male moths differ in their mate-finding capabilities and it is related to both pheromone response and morphological correlates. Male turnip moths *Agrotis segetum* (Dennis & Schiff.) were caught alive in pheromone traps in the field and tested the consecutive night in a wind tunnel. Males caught with high release rate baits in the field were responding significantly less in a wind tunnel to a low release rate bait, compared to males caught with a low release rate bait. No differences were found between males caught with a pheromone bait with a distorted ratio and males caught with a high release rate bait. The response time, i.e. time to induced behavioural response, also differed, but not the flight time, i.e. time to reach pheromone source from take-off. The morphological measurements revealed no differences in wing span, wing area or wing symmetry, but the weight differed between males typed low release-responding and males typed high release-responding. The last group was significantly heavier.

10-168

MILKWEEDS AND MONARCH BUTTERFLIES AND THE PARADOX OF PLANT CHEMICAL DEFENCES THAT BENEFIT SEQUESTERING SPECIALISTS

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Toxic chemical defences can pose a paradox for plants because many adapted insect specialists benefit from sequestration of these same chemicals for defensive use against their own natural enemies. Thus monarch butterflies, *Danaus plexippus*, increase their defence effectiveness as milkweed host plants increase investment in a group of toxic steroids called cardenolides. However, some highly modular milkweeds have constitutively low levels of cardenolide that can be induced extremely rapidly in a latex delivery system. Here we investigate experimentally the possibility that the latex delivery system helps to resolve this paradox through decreased growth of a specialist, sequestering herbivore. Our results agreed significantly with predictions that plant latex should decrease growth rates and increase mortality of first instar larvae of *D. plexippus*. A second experiment showed that milkweed cardenolides can be induced both quantitatively and qualitatively in ways that may also help to resolve the paradox.

10-169

BUMBLEBEE COMMUNITIES AND FLOWER VISITS IN EASTERN AUSTRIA (HYMENOPTERA: APIDAE)

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Bumblebee communities usually consist of many species coexisting within small areas. Mechanisms of resource partitioning were studied in 7 sites in a nature reserve in Eastern Austria using permanent study transects. Within one investigation period (May-October 1994) 10 bumblebee species (*Bombus* Latr.) and 3 cuckoo bumblebees (*Psithyrus* Lep.) were recorded.

The foraging dynamics of the species and their change in numbers in the frame of available resources were investigated with special reference to phenological patterns of the food plants. The 13 species visited altogether 37 plants from 12 families. The analysis of the food resources showed clear preferences of the *Bombus* and *Psithyrus* species for different flower types, depending mainly on the length of the tongue in relation to the length of the corolla tube.

10-170

SCARAB DUNG BEETLES ASSOCIATED WITH THREE SPECIES OF WILD MAMMALS IN THE QUEEN ELIZABETH NATIONAL PARK (UGANDA)

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A two-month sampling of dung beetles has been carried out in a savannah ecosystem of the Queen Elizabeth National Park (Uganda) by gathering droppings produced by three wild mammals: African Buffalo (*Syncerus caffer*), Hippopotamus (*Hippopotamus amphibius*) and Warthog (*Phacochoerus africanus*). Two kinds of habitats were considered: (1) dry soil savannah (in Mweya peninsula), and (2) muddy soil savannah (on the shore of Lake Edward).

A number of 2817 scarab beetles has been collected on the whole (71.8% Scarabaeidae, 28.1% Aphodiidae); the first family is more abundant in warthog (49.3%), the second one in buffalo dung (46.3%). Buffalo and warthog dung show both a higher value of beetle species diversity and biomass, respect than hippopotamus dung. As to different kinds of habitat, a lower value of scarab beetle biomass was calculated on muddy soils.

There is not evidence that any of the 26 species of Scarabaeidae will solely occur in one dung types, nevertheless many of them show a clear preference for one kind of food source. Two trophic groups were defined: (1) a group of thirteen species which prefer warthog, and (2) a group of nine species which prefer buffalo dung; none shows a significant preference for hippo dung.

The adults of two K-selected species belonging to two closely related genera of "rollers" (*Sisyphus crispatus* and *Neosisyphus spinipes*) show a phenological displacement. Indeed, the maximum values of density of these two species have been observed at a distance of one month each from the other.

10-171

DIVERSITY AND DYNAMICS OF A DUNG BEETLE COMMUNITY FROM A TYRRHENIAN COASTAL ECOSYSTEM

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The species composition and population dynamics of three different dung beetle communities were investigated in a protected area along the Tyrrhenian coast near Rome (Castelporziano). Dung beetles associated with wild board (*Sus scrofa* L.) have been examined during one year; those with horse and cow dung during two years.

The study area is a grassland surrounded by deciduous forest and evergreen scrub. A monthly sampling of each kind of dung was carried out to assess the seasonal change in both species composition and abundance of the beetle community. The results are discussed in relation with macro- and microclimatic data from the area and a comparison made with other ecological studies in Tyrrhenian coastal zones.

Out of the 51 species collected, 8 were exclusively found in cow, 2 in horse and 1 in wild board dung. According to Shannon-Wiener Index, April shows the highest peak of species diversity in both cow and wild board dung, and May in horse dung. The lowest value was observed in December-February. Concerning the seasonal succession of species and their relative abundance in the pasture, the highest value is in May and the lowest in December.

In the second year's sampling, results show a substantial stability of the community and the few variations may be explained by microclimate difference.

10-172

THE STRUCTURE OF THE ECOTONAL COMPLEXES
OF CARABID BEETLES (COLEOPTERA, CARABIDAE)

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The ecotonal complexes of carabid beetles have
been studied in four different ecotones on the
territory of the Berezinsky biosphere reserve
by means of pitfall trapping.

It was found that the ecotonal complex may
include four groups of carabid beetles:

- 1, 2 - species that live mainly in one of the
bordering ecosystems;
- 3 - species that are common for both
ecosystems;
- 4 - species that are found mainly in
ecotone.

The percentage ratio of these groups is not
constant. It differs in various types of
ecotones. In some ecotones one or two of those
groups may be absent.

10-174

COMPARATIVE INVESTIGATIONS ON CARABID FAUNA IN A
INTENSIVELY AND A EXTENSIVELY CULTIVATED VINEYARD
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1994 in a 0,5ha vineyard which hat been fallow for 2
years was ecological cultivation was established. During
the time of establishment the effects on vegetation and
fauna were investigated and compared with a intensively
used and treated vineyard. Major attention was payed to
the ground beetles (Coleoptera: Carabidae). 12 pitfall
traps were emptied all the year round on both acreages.
On the vineyard under ecological cultivation 43 species
occcured of which 9 were endangered and one species,
(*Acupalpus interstitialis* Reitter) first time recorded
for Germany. The intensively used vineyard was habitat
to only 16 species.

10-173

REPRODUCTIVE AND K-FACTOR LIFE TABLES OF THE
STRAWBERRY BLOSSOM WEEVIL, *ANTHONOMUS RUBI*
(HERBST) (COLEOPTERA: CURCULIONIDAE)

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Our studies in the population ecology of the strawberry blossom weevil
(SBW) have been developcd since 1977. 3 reproductive and
20 k-factor life tables of natural populations of SBW on strawberry
were constructed. It was found that the rate of development and the rate
of oviposition was greatest at 27°C, but fecundity - at 16°C. The net
reproduction rate (R_0) at 16, 20 and 27°C was 22,2, 19,4 and 11,4
respectively. The approximate intrinsic rate of increase (r_m^*) at optimal
thermal conditions (16 and 20°C) was 0,08-0,09 per day. Its value
depended greatly on mortality of individuals. In general, survival of
males was greater than females.

In 85 % cases females put only one egg in a flower bud, in 13,5 % cases
they put two eggs and in 1,5 % cases - three-five eggs in a bud. Some
females did not cut buds with eggs and the offspring was doomed to be
dead. There was some correlation between the number of double eggs
and population density ($r=0,634$, $n=17$).

K-factor life table studies showed that survival from egg to adult
emergence was very high (about 40-60 %), but total mortality from egg
to new egg was typical for insects (95-99,5 %). At the egg stage the
interruption of stereotype of females behaviour during oviposition was
the main mortality factor ($k=0,08-0,09$). The larval and pupal mortality
was mainly caused by parasitoids *Bracon intercessor* Nees, *Pteromahus*
(*H.*) *grandis* Walker, *Trichomahus* sp., *Lestodiplosis* sp. and sometimes
by invasion of bacteria *Pseudomonas* sp. We could not find proven
method for predicting the success of natural agents. The recognition of
the key factor (or factors) in autumn and winter was also an open
question.

The reproductive strategy of SBW will be discussed.

10-175

RELATIONSHIP BETWEEN NUTRITIONAL AND
THERMAL REQUIREMENTS OF *Trichogramma*
pretiosum RILEY, 1879, REARED ON DIFFERENT
HOSTS.

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The biology of *Trichogramma pretiosum* was studied
on *Anagasta kuehniella*, *Corcyra cephalonica* and *Sitotroga*
cerealella at six constant temperatures (18, 20, 22, 25, 30 and
32°C), in order to determine the parasitoid thermal
requirements when reared on these hosts. The thermal
requiriments was calculated based on hyperbole method. The
life cycle of this parasitoid increased as the temperature
decrease in all hosts tested. The life cycle of *T. pretiosum* was
shorter when reared on *A. kuehniella* than on *C. cephalonica*
and *S. cerealella*. The thermal constant was higher when the
parasitoid was reared on *C. cephalonica* (159,77 day-degree)
and *S. cerealella* (156,26 day-degree) than on *A. kueniella*
(129,39 day-degree). The lower temperature threshold was
10.05; 10.51 and 11.77°C when the parasitoid was reared on
C. cephalonica, *S. cerealella* and *A. kueniella*, respectively.

10-176

EFFECT OF LINEAR FURANOCOUMARINS AND ULTRAVIOLET LIGHT ON FEEDING BEHAVIOR OF *SPODOPTERA EXIGUA* (HUBNER) LARVAE

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Furanocoumarins (benz-2-pyrone compounds with a furan ring) are photoactive secondary substances; restricted in distribution among plants and appear to play a defensive role against herbivores. These compounds, like other allomones, evoke widely varying responses in different species of insects. Several reports indicate that furanocoumarins can act in a defensive role through modification of herbivore behavior.

Spodoptera exigua (Hübner), is an important pest of *Apium graveolens* L. (celery). Studies have shown the toxic effect on *S. exigua* of three linear furanocoumarins (bergapten, xanthotoxin, and psoralen) commonly found in *A. graveolens*. Furthermore, it has been suggested that on *A. graveolens* plants, late instar *S. exigua* larvae may be avoiding photoactivated furanocoumarins present in the more nutritious leaves by feeding within the less nutritious petioles which are exposed to lower ultraviolet light levels. However, there are no available studies documenting the effect of linear furanocoumarins, ultraviolet light (UVA) and their interaction on larval behavior of *S. exigua*.

Low concentrations of psoralen, xanthotoxin, and bergapten commonly present in healthy plants caused avoidance by larvae of *S. exigua* in choice tests with artificial diet. The effect of UVA light on larval feeding behavior and its interaction with furanocoumarins will be discussed.

10-178

STUDY ON ALARM PHEROMONE AND SEX PHEROMONE OF APHIDS

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Aphids are key pest insects in China with about 1500 species. (E)- β -farnesene (EBF) is the main component as aphid alarm pheromone. There were very different amount of EBF in homogeneous of different aphid species, but there was not found EBF in the bodies of *Brevicorye brassicae*. The primary rhinarium of the antenna of *Myzus persicae* was the main part for response to EBF. EBF possesses coordination effect with insecticide, it may reduce insecticide dose in the plant protection. EBF is stable enough to light, 55°C temperature, air bubble and in the mixture with insecticide, the half life were 13.3, 50.3, 21.3 days and 153 weeks respectively.

Nepetalactol and nepetalactone were two main components of sex pheromone in some species of Aphids. The attractiveness of the ratio 1:1 and 2:1 mixture were better in field traps to *Myzus persicae*. Some other species of aphids (including wheat ones) were also attracted and the attractiveness to some species of parasitoids was also observed.

10-177

LIRIOMYZA TRIFOLII (BURGESS) (DIPTERA: AGROMYZIDAE) PROBING, OVIPOSITION, DEVELOPMENT AND MORTALITY RATES AS AFFECTED BY COMMERCIAL COS LETTUCE (*LACTUCA SATIVA* L.) CULTIVARS

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The cultivar 'Tall Guzmaine' (TG) was preferred by *L. trifolii* adult females in choice tests 2.2:1 to 5.5:1 over other cultivars based on stipple counts (puncture wounds in the leaf surface). Preference for TG was maintained in no-choice tests. More probing occurred on all cultivars on the most recently fully expanded leaves in the middle of the plants than on leaves toward the bottom or top of the plants. Adult longevity and oviposition rates were greater on TG than on other cultivars in no-choice exposures. Development rates for egg through larval, prepupal through pupal and egg through pupal development periods were affected by cultivar. Females (342.3 ± 0.8 h) took slightly longer to develop from egg to adult than did males (339.5 ± 0.8 h). While cultivar did not affect egg and larval mortality rates, prepupal plus pupal mortality on 'Parris Island Cos' (24.3%) was 2.5 times greater than rates observed on other cultivars. Differences among cultivars in all the tested parameters appears to closely follow the pedigrees of the tested cultivars. Probing preferences, shorter development and lower mortality rates for *L. trifolii* on cos lettuce were associated with 'Paris White' in the cultivar's pedigree.

10-179

ISOZYME ANALYSIS OF ESTERASE, ALKALINE PHOSPHATASE, LEUCINEAMINOPEPTIDASE, ALCOHOL AND OCTANOL DEHYDROGENASE DURING LARVAL DEVELOPMENT OF *Anastrepha fraterculus* (DIPTERA: TEPHRITIDAE).

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The fruit fly *Anastrepha fraterculus* is a major pest of native and exotic tree fruits in Brasil. Ecological, ethological and evolutionary aspects has been well studied. However, developmental biologic aspects studies are still incipient. In this work, we characterized the isoenzyme patterns of esterase (EST), Alkaline phosphatase (APH), Leucinoaminopeptidase (LAP), Alcohol and octanol dehydrogenases (ADH, ODH) in larval development in polyacrilamide gel. The samples analysed were rearing under laboratory conditions (14L:10D, 25°C, 70%RH) in papaya fruit. The results showed a dynamic variation of isoenzymes expression. We observed five EST eletromorphs, four APH eletromorphs and 12 eletromorphs to ADH and ODH dehydrogenases. Probably studies in *A. fraterculus* geographic populations using isoenzymes related with insect development can help to elucidated the speciation question because this species is considered as complex of criptical species that explore wide range of hosts in several environmental conditions. Grants and Fellowships: CNPq, ABPM.

10-180

Drone Flight Time of Tropical Honeybees and the Influence of Light on the Drone Flight Behaviour

by

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Abstract

The time occurrence of drone flight activity of the AsianTropical honeybees (*Apis cerana*, *Apis andreniformis* and *Apis dorsata*) colonies were monitored at several locations in Malaysia and Indonesia to investigate the factors influencing drone flight or mating flight activity

There were distinct differences on the occurrence of peak times and duration span of drone flight activities between the honeybee species at different locations. The drone flight activity is very much linked to the local azimuth. There is also the influence of light as the experimental on-off manipulation of a fluorescent light bulb (one-sided illumination) on the colony of the open-nesting *Apis dorsata* suppressed drone flight activity.

10-182

STUDIES ON THE POPULATION DYNAMICS OF *CHORTHIPPUS BUBIUS* (ZUBOVSKY)

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Based on data from systematic investigation on the alpine prairie, Xiahe County, Gansu Province, the simulation equation of the growth and decline of numbers of *Chorthippus dubius* (Zubovsky) was established. The spatial pattern and dynamics of the population were analysed by calculating five indices of aggregation, parameters of Taylor model and Iwao model. The results showed that the spatial pattern of 1-2 instars belonged to an aggregated distribution, but that of 3-5 instars and adults became random or uniform. The theoretical formula for determination of the sampling number was given. The sequential sampling was analysed. It is better to combine Iwao's sequential sampling method with Kuno's one.

By using the average density, the index of patchiness, and the frequency of plot existing insects, the population dynamic of *Chorthippus dubius* was studied by the method of fuzzy cluster analysis and then divided into the initial stage of the hatching and last stage of the adults, the low instar stage of the nymphs, the mixed stage of the nymphs and adults, the adult stage.

10-181

COMMUNITY ORGANISATION AND DIVERSITY PATTERNS OF THE GALL-FORMER COMMUNITY ON COMMON REED

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Internally feeding herbivore insects are strongly dependent on the growth characteristics of their host plants. Moreover, insect induced plant galls usually form a base for a complex community of parasitoids and predators. Therefore, they are ideal objects to study the mechanisms of community organisation along several trophic levels. The flies of the genus *Lipara* Meigen (Diptera: Chloropidae) are strict monophagous parasites of the common reed, *Phragmites australis*, upon which they induce typical cigar- or spike-like galls. Dependant on the growing conditions, a reedbed can comprise shoots from a wide diameter range. Previous results showed that the diameter of a particular reedshoot is very important for the success of the *Lipara* herbivores. When *Lipara* galls are opened, several larvae turn out to be parasitized by Hymenoptera, while other are killed by predators.

To analyse the interactions between the species of the different levels in the community, a field experiment was carried out. During winter, when all species are in diapause, galls were collected at different localities in Belgium and the Netherlands by means of 0.25 m² quadrat samples. The galls were dissected and the inhabiting species (*Lipara*, parasitoids, predators) were identified. Additionally we measured soil and vegetation characteristics and reedshoot diameter and density. Community structure, species diversity and interaction between the species were analysed with structural equation modelling.

Both the number and the diversity (e.g. Shannon-Wiener) strongly depend on the reedshoot diameter distribution and soil moisture of a reedbed. The mortality due to parasitoids and predators is highest for *L. rufitarsis* and *L. lucens*. However, in many localities, parasitoids are completely absent. The different parasitoids and predators show a specific host spectrum. Parasitoid and predator diversity increase when the overall *Lipara* gall density increase and reedshoots are thinner. Both are independent of *Lipara* species composition and diversity.

10-183

INTERACTION BETWEEN SEX RATIO AND POPULATION DYNAMICS IN SAWFLIES (HYMENOPTERA: SYMPHYTA) K. Otsuka

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A study of a tenthredinid sawfly, *Strongylogaster osmundae*, revealed that some females deposited eggs without mating first in the field. On the other hand, mated females deposited more female than male eggs. This behavior agreed qualitatively with an ESS (evolutionarily stable strategy) model. Mated females produced offspring with a more female-biased sex ratio than predicted by the model when the population density and proportion of mated females were high. This seemed to be caused by low phenotypic plasticity.

Based on the above observations, a simulation model of sex ratio and population dynamics was devised. It was assumed that sex ratio genes on one locus determine the sex ratio of offspring of a mated female, and that the proportion of mated females is high when the population density is high.

This model suggested that:

- 1) The sawfly (haplo-diploid) population sex ratio becomes female-biased than 1:1 when a population outbreak occurs.
- 2) The probability of extinction is lower and probability of a population outbreak is higher in a haplo-diploid than in a diploid population.

10-184

ON INTERFERENCE BETWEEN INSECT PARASITIDS IN A PATCHY ENVIRONMENT

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Interference between insect parasitoids can be viewed as the consequence of changes in behaviour in response to increasing levels of intra-specific competition. It can serve as a stepping stone from individual behaviour to population dynamics. We present an expression for the overall search rate for a habitat where hosts have a patchy distribution and where there is intra-generation travelling between patches by the parasitoids. We describe two well-known forms of interference: mutual interference and pseudo-interference, and also a more recently proposed form, indirect mutual interference. This type of interference is caused by changes in behaviour at different parasitoid densities that affect the time budgets of the parasitoids or the distribution of parasitoid effort over patches. We present some general methods to distinguish between the different forms of interference and discuss the difficulties in applying these methods to multi-patch laboratory experiments and field data. Next, these methods are applied to a multi-patch experiment using the parasitoid *Trybliographa rapae*. In these experiments, either 1, 5, 10, 15 or 20 parasitoids are released in an arena with 5 patches. Hosts are either distributed evenly (12 each) or aggregated (2, 4, 8, 16 & 32) over these patches. All arrivals and departures are registered over the 8 hours the experiments ran. Overall search rate decreased with parasitoid density for both host distributions. With an increasing number of parasitoids in the arena, a linear increase in the mean and a decrease in the coefficient of variation (CV) of the distribution of patch times over patches was found. Thus, pseudo-interference and indirect mutual interference plays a role.

10-186

STUDIES ON THE ECOLOGY AND BEHAVIOUR OF *Plutella xylostella* (L.) (LEPIDOPTERA: PLUTELLIDAE). MORTALITY AND DISPERSION.

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Plutella xylostella is an important pest of cruciferous crops. Experiments were carried out in controlled environment rooms (20±1°C constant temp., 16 h/day light, rel. hum. not controlled was 44-52% by day, 14% higher at night) to determine development, mortality and dispersion of populations of *P. xylostella* in conditions where all external factors were similar except the initial density of the eggs or larvae and the quality of the host plant. Cabbage plants grown in greenhouse were used. Leaves were from the plant medium region neither senescent nor part grown. The area of the leaves varied from 10 to more than 300 cm². Small transparent plastic cages were used to confine females to selected places where to lay eggs, during one day. Eggs laid anywhere except the upper surface of the leaves were removed. Eggs density varied in different experiments: 1 to 641 per plant, 1 to more than 400 per leaf, 0.5 to 130 per 100 cm²; some were isolated, others in pairs close or separated, or in groups of 10; 35-50; 110-120 per leaf (one or four leaves with eggs per plant). The plant age and the leaves condition (tenderness/toughness) varied. Egg mortality was very low (around 5%), probably due to infertility. Most of the mortality occurred in early stages of the larvae (first and second instars) especially during the first week of life when start to disperse; probably need more space per initial larvae to burrow galleries. Recently hatched larvae disturbed each other and some fell onto the ground or did not established feeding place and die. There was no pupal mortality. There is intra-specific competition of the young larvae at high density (density dependent). The later larval stages did not suffer notable increases in mortality. Intra-specific competition does not affect pupation in terms of survival of adult stages. In tender leaves the distance from egg to first feeding place was 9.5 ± 2.03 mm in eggs laid isolated and 17.6 ± 1.07 mm in eggs laid in groups. The overall pattern of dispersion is affected by the initial density of the larvae and the mortality of the early instars. Higher the density: higher the mutual disturbance and higher the early instar mortality, and the larvae take shorter time to get evenly distributed over the leaves. At lower densities mortality is lower and the larvae (especially early instars) remain longer in their original feeding place, maintaining a similar level of aggregation for longer time. As larvae grow bigger, disperse more evenly throughout the different leaves of the plant. There is little movement from plant to plant. The larvae tend to remain in that even distribution up to the end of the larval stage when tend to aggregate before pupation. This is also shown by aggregation indices.

10-185

COMPARATIVE STUDY OF BEHAVIOR IN ITALIAN AND AFRICANIZED *APIS MELLIFERA* SUBSPECIES : GROOMING AND REMOVAL BEHAVIOR AS AN ELEMENT TO EXPLAIN THE DIFFERENTIAL POPULATION DYNAMICS OF *VARROA JACOBSONI* MITER. VANDAME^{1,2}, M.-E. COLIN¹, G. OTERO-COLINA²¹ Institut National de la Recherche Agronomique, Station de Phytopharmacie, 84143 Avignon, France² Colegio de Postgraduados, Instituto de Fitosanidad, Campus Córdoba, 94500 Córdoba, VER., Mexico

In tropical climate of South Mexico, the africanized *Apis mellifera* honey bee subspecies was found as resistant to *Varroa jacobsoni* parasite, whereas italian honey bee was found as susceptible.

Following the descriptions of Peng (1987), a study of grooming behavior in these subspecies revealed that both types of bees had a high level of auto- and allo-grooming behavior. However, the result of such a behavior staid very poor, since less than 20% of observed mites changed bees and less than 10% failed from bees.

Consequently, the mutilation rate of naturally dead mites was very low, about 15% in africanized colonies, and 10% in italian colonies. A study of removal behavior of infested brood showed that between fifth and seventh day of capping stage, africanized bees removed more than 30% of parasitized brood, while italian bees removed only 10% of parasitized brood. Such a difference could be an important element to explain the differential population dynamics of the mite between resistant and susceptible honey bee colonies.

10-187

COPING WITH COLD: ARE UPLAND POPULATIONS OF THE CRANE FLY *TIPULA MONTANA* CURTIS (DIPTERA: TIPULIDAE) BETTER ADAPTED THAN LOWLAND COUNTERPARTS?R.M. Smith¹ and C.M. Todd²¹ Department of Zoology, University of Aberdeen, Aberdeen, UK. ² British Antarctic Survey, Cambridge, UK.

Tipula montana Curtis is adapted to alpine and boreal biotopes and it is freeze tolerant in the overwintering larval stage (instars 2, 3, 4). Aspects of cold tolerance were compared between a mountain population (1000m a.s.l.) and an atypical population in moorland (400m a.s.l.) to test the hypothesis that adaptation to cold declines in less severe winter environments.

Cold tolerance was assessed in each instar by measuring the concentrations of cryoprotectants (with gas chromatography), lower lethal temperatures, and larval survival at different sub-zero temperatures. The effects of cold stress upon adult fitness were investigated by measuring adult body size, female fecundity, and survival.

10-188

HOW INSECTS DIE: DEMOGRAPHIC TRENDS IN FIELD POPULATIONS

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Quantitative estimates of mortality for 86 phytophagous species of Coleoptera, Diptera, Lepidoptera and Hymenoptera were extracted from published field life tables and tested for differences associated with the developmental stage and five ecological characteristics of pre-adult herbivores (feeding biology, invasion status, and the successional stage, cultivation status, and latitudinal zone of the habitat). Mortality rates were most strongly associated with the developmental stage and feeding biology of herbivores. The remaining variables showed little or no relationship with mortality. Across all species and life stages, natural enemies kill the most herbivores and competition the least.

10-190

INFLUENCE OF PHYSIOLOGICAL, ENVIRONMENTAL AND GENETIC FACTORS ON LONG DISTANCE FLIGHTS OF *CYDIA POMONELLA* L. (LEPIDOPTERA: TORTRICIDAE) MEASURED BY A FLIGHT MILL

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Cydia pomonella L. (Lepidoptera: Tortricidae) is a global pest of apples. Long distance flights of *C. pomonella* are believed to be important for its colonisation of new habitats, and may also help to maintain genetic variability within the population. In the field, long distance flights up to 11 km have been reported for male codling moths. More knowledge of the factors that influence this behaviour is particularly important for the implementation of the pheromone confusion technique. In this study the flight capacity of *C. pomonella* was measured in the laboratory by using computer linked flight mills. The ability to undertake long flights (defined as continuous flights longer than 5 km) was uniform amongst different populations within northern Switzerland, but significantly reduced for moths of our laboratory colony. We found only small differences between the sexes in the distances that they would fly. The distance flown increased during the first 3 days after emergence (20°C) and then decreased continuously until death (around age 20 days). This decrease was stronger for mated than for virgin moths of both sexes. The ambient temperature was one of the most important environmental factors influencing flight distance. The strongest decrease occurred at 20°C to 15°C. Part of the variation in flight distance was found to be genetically determined. We suggest that there may be a genetic trade-off between flight distance and fecundity.

10-189

THE CONSEQUENCE OF THERMOREGULATION FOR LIFE HISTORY AND POPULATION DYNAMICS OF THE GRASSHOPPER *STENOBOTHRUS LINEATUS* (PANZER) (CAELIFERA: ACRIDIDAE)

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Between 1992 and 1995 the population dynamics of *S. lineatus* was investigated on a semi-arid calcareous grassland in Thuringia/Germany. A key factor analysis indicated the time interval between reproduction of the females and hatching of the first instar nymphs as decisive for the observed population dynamics. To analyse the causality of dynamics, the habitat structure, the microclimate and its effects on the species were investigated.

The body temperature of females was recorded in field studies between 1993 and 1995. The results show significant differences between the ambient air temperature and the thoracic temperature of the grasshoppers, depending on the solar radiation energy. Some females of *S. lineatus* achieved differences up to 20 degrees by basking in the sun.

To investigate the consequence of this heat-up for life history, a field experiment to oviposition frequency and pre-oviposition period of *S. lineatus* was conducted. Furthermore, comparative laboratory experiments were conducted by single keeping of *S. lineatus*-couples on standardized conditions of diet, temperature, light and radiation energy.

In the field a mean oviposition frequency of 0.4 pods per day was recorded at an ambient temperature of about 21°C and a radiation energy of 700-900 W/m². In the laboratory at cold light (below 200 W/m²) the same value of oviposition frequency was not achieved until a mean daily air temperature of 35°C. Due to the heat-up, a multiple increase of oviposition frequency could be proved. Thus, at Central European temperate climate the solar radiation energy in the habitat structure and the behaviour of thermoregulation allow the reproduction of the species at all and have a decisive consequence for the population density in the following generation. Furthermore, as an important contribution to life history data and a detailed key factor analysis, the results of the experiments allow to calibrate the oviposition frequency to the actual weather conditions between 1992 and 1995 and to re-estimate the number of laid eggs within four generations of *S. lineatus*.

10-191

SIZE OF EGG CLUSTER, FEMALE SURVIVAL AND LIFE-TIME EGG PRODUCTION, IN THE HERBIVORE BEETLE *GALERICUCELLA SAGITTARIAE* (CHRYSMELIDAE) IN NATAL AND NOVEL HOST PLANT *POTENTILLA PALUSTRIS*.

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I conducted a laboratory experiment to investigate several life-history parameters of a herbivore-beetle, *Galericucella sagittariae*, in natal and novel *Potentilla palustris* plants. In the experiment I compared number of egg clusters, number of eggs per cluster, total number of eggs laid per female and adult survival in the two different localities. No significant differences were found between natal and novel plants. However, an inverse relationship between cluster size and female longevity were found. This experiment does not seem to support the generally accepted hypothesis that herbivores are adapted to natal plants but maladapted to colonize novel conspecific plants (deme-formation hypothesis).

10-192

ECOSYSTEM IMMUNITY OF A BIOTIC COMMUNITY AGAINST PEST INSECTS

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Populations of insect pest sometimes grow up to the high level of economic problem, but usually they are maintained at low level below the economic injury in nature.

Living bodies have a system of immunity against pest invasion, such as macrophages etc. in human body to suppress pest organisms below the level to become illness. In this case, macrophages are selfperpetuating in human body at an expense of blood and body fluid. Analogically crop populations have a defense mechanism in the biotic community against pests by means of natural enemies as like as an immunity in a total system. However, natural enemies, parasites and predators, must be maintained themselves without any support of agroecosystem.

Many years ago I proposed a model 'Reproduction curve with two equilibrium points' to consider the fluctuation of insect population (Takahashi, 1964). The lower equilibrium point in the model can be regarded as a latent period and another higher one as an outbreak level. This model is based on a S-shaped functional response of predator to prey density and is applicable in a biotic community where polyphagous predators predominate.

To keep stable population of natural enemies in the biotic community, it is necessary to maintain their food stability in the fields. In the monophagous predators such as parasitoids, their populations fluctuate in response to prey or host populations sometimes inducing violent fluctuation of pests over the economic injury level. On the other hand, in the case of polyphagous predator, they can find ordinary organisms as alternate foods and keep their population stable even when a pest population, a target food, decreases to low level. This model will be discussed with respect to the diversity of biotic community.

10-194

HETEROGENEITY AND DENSITY DEPENDENCE IN A FIELD STUDY OF A TEPHRITID-PARASITOID INTERACTION

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The spatial distribution of tephritid flies attacking thistle flower heads and the levels of parasitism from the associated parasitoid guilds were studied over a number of years.

Recent developments in population ecology have shown how the effects of spatial heterogeneity of parasitism in a wide variety of discrete-generation host-parasitoid models can be assessed using a simple rule. Specifically, this states that the coefficient of variation squared ($CV^2 = [\text{variance}/\text{mean}^2]$) of the density of searching parasitoids in the vicinity of each host must typically exceed unity for the heterogeneity in parasitism to be a sufficient cause of stability in the interacting populations.

This data set provides a rare opportunity to use the methods of analysis outlined above to seek both temporal, density dependent relationships between average levels of parasitism and host density per generation, and also any spatial patterns of parasitism contributing to stability that may be operating within the same field system.

10-193

REPLICATED, LONG TERM, HOST-PARASITOID POPULATION DYNAMICS

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Replicated, long term population studies provide the foundations against which hypotheses on the mechanisms of regulation can be accepted or refuted. In this study, data from replicated, host-parasitoid laboratory interactions (*Plodia interpunctella* - *Venturia canescens*) are analysed using various methodologies. The study identifies the underlying dynamics and explores the relative roles of density dependent and density independent processes within the interaction.

10-195

INDIRECT EFFECTS IN HOST-PARASITOID ASSEMBLAGES

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Hosts which share a common natural enemy may experience indirect (apparent) competition. Theory predicts that this effect should lead to the elimination of one of the host species. On the other hand, various mechanisms such as density dependent factors, predator switching and temporal refuges, have been suggested as mechanisms promoting coexistence between hosts sharing a common natural enemy.

In this study, apparent competition is explored, both empirically and theoretically, as a factor influencing coexistence in host-parasitoid assemblages.

10-196

MODELLING INSECT NATALITY USING SPLINES FOR POTATO LEAFHOPPER

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We present methodology for analyzing age-specific birth rate, with particular emphasis on describing variability within a cohort of like-aged individuals. We use a model structure appropriate for natality in insects natality and other arthropod species. Our approach separates natality into a baseline rate representative of the effects of the environment for the population and a factor that represents the unique effect of each individual in the cohort. We employ something splines with the penalized likelihood methodology for estimation. We illustrate the methodology by applying it to data on leafhopper egg-laying rates.

10-198

FACTORS AFFECTING THE PERFORMANCE OF PHEROMONE TRAPS FOR *SPODOPTERA LITTORALIS* (BOISD.), *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) AND *EARIAS INSULANA* (BOIS) IN KAFR EL-SHEIKH, EGYPT

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ABSTRACT

The present work was conducted at the experimental farm of the Agricultural Research Station, Sakha, Kafr El-Sheikh Governorate, Egypt, and lasted for two complete years. Its objectives were to record the populations of three insects infesting cotton "*Gossypium barbadense* (L.) and the Egyptian clover "*Trifolium alexandrinum* (L.). The pheromone trap was the main tool for capturing *S. littoralis* (Boisd.), *Pectinophora gossypiella* (Saunders) and *Earias insulana* (Bois). The effect of 3 main weather factors, max., min. temperature and R.H.% on the density of those pests was also under control. Annual generations for every pest was estimated after the recorded device by Audemard and Milaire (1975) and Iacob (1977). Results indicated the occurrence of 3 peaks per year for *S. littoralis*. Two peaks were recorded for *P. gossypiella* in 1994 and only one in 1993, while three peaks were clear per year for *E. insulana* in either 1993 or 1994. A clear relationship was recorded between numbers of *P. gossypiella* happening in autumn and infestation in the following cotton season and that phenomenon could be used as a prediction. Simple correlation between the 3 tested weather factors and pest catch were recorded, plus the effect of the 3 weather factors and determination of coefficient value. The number of estimated generations was 7/*S. littoralis*, 5/*P. gossypiella* and 5/*E. insulana*.

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INTRODUCTION OF A NEW WHITEFLY TRAP

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We designed a adult whitefly trap based on behavior observations that showed: 1) flight response toward light, 2) yellow color attractiveness, and 3) landing behavior on hosts followed by walking to undersides of leaves. Studies to evaluate the efficacy of the trap were conducted in the southwestern irrigated desert crop growing areas of the United States. The traps studies were conducted in five *Bemisia argentifolii* Bellows and Perring chemical control projects on cotton, *Gossypium hirsutum* L. and one chemical control project on cantaloupe *Cucumis melo* L., var. cantalupensis. The 1995 experimental plots were located at USDA-ARS Irrigated Desert Research Station at Brawley, CA and the University of Arizona Maricopa Research Center, Maricopa, AZ. Results showed that the new whitefly trap catch data reflect seasonal dispersal, population variations induced by chemical control, and seasonal population changes on cotton and melon. The numbers caught were comparable to numbers caught with a commercial trap designed for fruitflies using a bait. The new whitefly trap is a non-overloading, non-sticky, no bait trap. The new whitefly trap may be a useful detection, survey and monitoring tool.

10-199

AUTOMATED MONITORING OF FREE-FLYING INSECTS USING WINGBEAT WAVEFORMS

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A computer-based field instrumentation system uses an optical sensor to measure rapid changes in the intensity of light reflected from the wings of individual flying insects. Output from the sensor contains species-specific wingbeat waveforms that are automatically classified using artificial neural networks that have been trained to identify species of interest. The system can monitor many species within a given habitat. Potential applications in the areas of medical entomology and integrated pest management are discussed.

10-200

SIMULATION OF THE WHITEFLY-ENCARSIA FORMOSA INTERACTION, BASED ON FORAGING BEHAVIOUR OF INDIVIDUAL PARASITOIDS

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Greenhouse and sweet potato whitefly (*Trialeurodes vaporariorum* and *Bemisia tabaci*) are very common, highly polyphagous pest insects all over the world. Biological control of greenhouse whitefly with the parasitoid *Encarsia formosa* has been applied with great commercial success during the past 20 years, while natural enemies for sweet potato whitefly are now evaluated. Modelling has always played an important role in the process of selecting and improving the efficacy of releases of natural enemies, but often biologically unrealistic simplifications were part of these models which strongly limited their predictive value.

We have developed a model which is unique in that it is individual based and simulates the local searching and parasitization behaviour of individual parasitoids in a whitefly-infested crop. The model includes stochasticity and spatial structure based on location coordinates of plants and leaves. This model comprises several submodels for (a) the parasitoid's foraging behaviour, (b) the whitefly and parasitoid population development, (c) the spatial distribution of whitefly and parasitoid within and between plants in the crop, and for (d) leaf production. With the model we can simulate temporal and spatial dynamics of pest and natural enemy. The model will allow us (1) to explain why the parasitoid can control whiteflies on some crops and not on others in large commercial greenhouses, (2) to improve introduction schemes of parasitoids for crops where control was difficult, and (3) to predict effects of changes in cropping practices (e.g. greenhouse climate, choice of cultivars) on the reliability of biological control, and finally (4) to develop criteria for the selection of natural enemies.

10-202

DETERMINISTIC AND STOCHASTIC MODELS OF THE SHEEP BLOWFLY, *LUCILIA SERICATA*

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Deterministic and stochastic simulation models have been constructed to predict the seasonal pattern of abundance of the ectoparasitic blowfly, *Lucilia sericata* in sheep pastures. These models use empirically derived relationships between temperature and development rate. The number of day degrees accumulated each day by a cohort is calculated from the daily temperature pattern and the base temperature threshold for that stage. The stochastic model uses a Monte-Carlo simulation technique to assign random development rates to each life-cycle stage generated from a Weibull distribution fitted to observed variation in development rate for each stage. Sensitivity analysis has been carried out on the life history parameters in the models in order to determine the importance of the factors affecting the dynamics of the sheep-blowfly system. The results of this work and extension of the models to include farm management factors will be discussed.

10-201

MODELS OF STOCHASTIC DEVELOPMENT IN INSECT POPULATIONSG. Di Cola, G. Gilioli, A. Cossu¹

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Stochastic simulation models of single species insect population dynamics are proposed as basic components in pest prediction, management and decision making systems. The models describe the development process of an age-structured population taking into account recruitment, death, environmental and biological variability.

In these models the insect development process, as function of temperature history, is represented by a Brownian motion process with drift, described by a stochastic differential equation in terms of thermal units and other environmental and biological parameters. Random fluctuations due to the variable environment are introduced as stochastic element in the death rate. Since environmental changes are due to many factors, they are approximated with a stochastic differential equation describing the mortality in the population.

The numerical simulation of these processes is performed by means of the fractional step method in which, for every small time step, the random walk principle is used to model the diffusion term (related to the biological variability) combined with other development, recruitment and death mechanisms. At each time step, according to the position of individuals, represented by particle with mass (i.e. computational elements), the developmental level increases, in relation to the drift, of a certain quantity modified by an amount drawn from a continuous probability distribution. These processes are combined with the mortality which is modelled by destruction of computational elements with a given probability depending on death rate. The recruitment is described as an inward flux and leads to the creation of new computational elements on the boundary.

As a case study numerical simulation of *Bactrocera oleae* (Gmel.) populations development in olive groves has been performed. The results are compared and discussed.

10-203

GENSECT - A GENERIC APPROACH TO CONSTRUCTING POPULATION MODELSG.F. Maywald, R.W. Sutherst, S. M. Richardson and M.P. Zalucki
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Many biologists would like to use population models, either to test hypotheses, or to design management strategies. However, the construction of models is beyond the resources of most ecologists without access to a computer programmer. GENSECT provides a research, management and teaching tool that can be used to construct a model of any species without the need for programming.

A major consideration in the design of GENSECT was the provision of a user-friendly interface to make the process of constructing a model easy and enjoyable, with concepts being presented in language familiar to biologists. Different lifecycles are assembled from a 'toolbox' of components by attaching properties like development or survival rates to lifestage objects such as eggs, larvae or plant growth stages. Other components, such as microclimate models or data file readers, are also available as modules, and can be easily coupled to the lifecycle. All internal model variables are available for output as graphs or tables, thus simplifying the construction and error-checking of a new model. Models of different species can be saved as files, which will serve as an important means of summarising and exchanging information about a species' population dynamics. After the model is constructed, GENSECT provides a user-friendly environment in which to run the model, and display selected results.

10-204

CONSEQUENCES OF OBLIGATE KILLING ON THE POPULATION DYNAMICS OF ODNATES AND THEIR MOSQUITO PREY IN TROPICAL TREE HOLES

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Water-filled tree holes in the neotropics are a poorly known, but important habitat for a diversity of aquatic macrofauna. In a seasonal Panamanian forest, the larvae of four odonate species are top predators in this unique community. Experiments with *Megaloprepus coerulatus*, a giant damselfly, revealed that larvae kill conspecifics even when well-fed. Under field conditions where prey are scarce, cannibalism and intraguild predation reduces predator density to only 1-2 larvae/liter. Such regulation permits maximal larval growth, increasing the chance that the surviving larvae emerge before the habitat dries out seasonally. Given that the odonates reduce their own numbers to such low levels, what, if any, effect does this guild of predators have on the populations of tree hole mosquitos, their most ubiquitous prey?

In a field experiment using 0.4 l artificial holes seeded with a natural level of detritus, odonates reduced both the total number of mosquito larvae and those surviving to pupation, relative to controls. With high initial nutrient input (a small fruit), the total number of mosquito larvae did not differ between controls and holes containing an odonate predator. Surprisingly, in the treatment with *M. coerulatus*, the number of mosquito larvae actually increased, suggesting that this predator may chemically attract ovipositing mosquitos. Nevertheless, in all predator treatments, the number of mosquitos surviving to pupation was significantly reduced relative to controls. This result is explained by the finding that odonates preferentially ate the largest mosquito larvae. Our work suggests that the ultimate size as well as the behavior of the prey species determine the impact tree hole odonates have on these sylvan mosquitos.

10-205

LOCAL DYNAMICS OF TROPHIC INTERACTIONS IN ACARINE SYSTEMS

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A lumped parameter model representing the dynamics of interacting species is presented: the model is described by a system of O.D.E. in terms of biomass with few observable parameters, with the aim of reducing the complexity of the system and of supporting the practical use.

Methods are proposed for estimating demographic and physiological parameters related to birth, growth and death processes as well as to food consumption and conversion. For this purpose numerical simulations with single-species age-structured models have been carried out.

The dynamics of the system is characterized by the growth function of the prey, the functional response of the predator, and death functions for both populations; these functions depend on both ecophysiological and behavioural parameters.

Basic model properties are studied with particular reference to functional response functions. The model is used to study the dynamics of acarine systems, relevant to poultry houses and protected crops.

Section 11

Special Environments Entomology

11-002

EVOLUTION OF PATERNAL BROODING IN THE GIANT WATER BUGS
(HETEROPTERA: BELOSTOMATIDAE)

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Unilateral post-zygotic paternal care is extremely rare among animals. The giant water bug family Belostomatidae contains most of the arthropod species known to exhibit this unusual behavior. In the subfamily Lethocerinae, males brood eggs laid on emergent vegetation. Brooding in this group involves watering eggs, shading them, and defending them against predation. In the subfamily Belostomatinae, males employ a variety of behavior patterns to aerate eggs attached to their backs by their mates. Brooding is obligatory in all belostomatid species studied, unattended eggs invariably die if left in open air or submersed. This paper explores the biology, phylogeny, and fossil record of the Belostomatidae and related taxa in an attempt to discern the selection forces, constraints, and the sequence of historical events responsible for the evolution of this unusual behavior and its subsequent diversification. Selection for large bug size, in order to take advantage of vertebrate prey, together with the dual phylogenetic constraints of Dyar's Law and the apparent inability of heteropterans to add molts, coupled egg size to body size. Thus selection for large bugs also produced large eggs--too large to develop unattended submersed in water. A past history of eggs being laid in water left these larger eggs lacking the necessary adaptations to survive desiccation when laid unattended in open air. Consequently, large eggs created selection for an innovation to lift egg-size limitations on imago size. Ergo, emergent brooding evolved. The initial costs of emergent brooding were minimal for males who required less food than females, possessed a perfected anti-cuckoldry mechanism, and who were able to obtain multiple mates and brood multiple clutches of eggs. Females in contrast, would have sacrificed substantial future fitness in order to brood eggs and were therefore selected to abandon. Transitions from non-brooding to emergent-brooding and back-brooding are inferred under different assumptions about the behavior of members of the enigmatic genus *Horvathinia*. The evolution of back-brooding was followed by an adaptive radiation. Water bugs in the fossil genus *Mesobelostomum* from the Upper Jurassic probably brooded their eggs more than 150 million years before present. Giant water bug brooding, although complex as expressed in extant species, may have represented a modest innovation at the time of its origin. However, this behavioral innovation lifted constraints, and thus permitted the evolution of larger bugs able to prey on larger aquatic vertebrates, shifting the lineage to new adaptive peaks. Although existing general theory on parental care could not have predicted brooding in the Belostomatidae, much of the theory on unilateral postzygotic paternal care, fits giant water bugs very well. The term "ancillary selection" may usefully distinguish natural selection which produces characters supportive of primary traits. Brooding in the Belostomatidae is deemed to be a product of ancillary selection which supports primary selection for large bug size to facilitate the use of vertebrate prey.

11-003

EVOLUTION OF SECRETION-GROOMING AND
RESPIRATORY HAIR STRUCTURES IN NEPOMORPHA

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Some water bugs from time to time crawl onto land to spread the secretion of their metathoracic scent glands over their water-repellent pubescence („secretion-grooming“). The pubescence is enclosed by an air sheath when submerged and has a respiratory or hydrostatic function. Experimental prevention of secretion-grooming leads to the loss of the respiratory bubble and a significant increase in mortality. The antimicrobial secretion probably keeps the pubescence free of bacterial contamination and thus hydrophobous and functional.

The secretion-grooming behaviour can be released experimentally by an increase in water temperature or light intensity. This method was used for a comparative study of the secretion-grooming patterns and to test, whether secretion-grooming occurs in all families of Nepomorpha. In all, 45 species (28 genera) from Europe, Central America, Southeast Asia, Australia and New Zealand were studied.

It was found that the occurrence of secretion-grooming is correlated with the architecture of the hydrofuge hairs and their presence or absence, respectively. Secretion-grooming with different grooming patterns occurred in five out of nine families: Corixidae, Naucoridae, Notonectidae, Pleidae and Helotrephidae. A model is presented on how secretion-grooming may have evolved in Nepomorpha.

11-001

THE EVOLUTION OF MATING SYSTEMS AND SEXUAL
DIMORPHISM IN WATER STRIDERS (HETEROPTERA,
GERRIDAE)

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Water striders are conspicuously adapted for life on the water surface and their two-dimensional habitats make them ideal objects for behavioral studies. Matings in water striders can be divided into two distinct types. In type I matings, males search actively for mates and when females are encountered, males initiate matings by simply lunging at females, without prior courtship. Typically, females struggle vigorously before allowing intromission. On the other hand, males usually exhibit post-copulatory mate guarding. In type II matings, males are typically territorial, defending suitable oviposition sites, and "court" females by producing surface ripple signals.

Sexual dimorphism is usually very pronounced in water striders. Several authors have proposed that, given sexual conflicts over mating, male and female genitalia and other structures may be involved in a coevolutionary arms race. This hypothesis predicts that males should evolve structures to cope with female resistance and that females should evolve structures to resist male harassment and gain increased control over matings.

So far, studies of insect mating systems and sexual selection have chiefly been focused on single species or on comparisons among species without attention to their phylogenetic relationships. However, phylogenetic data can contribute significantly to studies of insect ecology and behavior by permitting inferences about the evolutionary history of traits. Here, this approach is applied to the monophyletic group Gerridae, focusing on the evolution of sexual dimorphism in relation to mating systems and the coevolution of male clasping devices and female antilasper devices.

11-004

AQUARIUS REMIGOIDES: A NEWLY-DESCRIBED, LARGE AND ABUNDANT WATER STRIDER FROM THE SOUTHEASTERN UNITED STATES.

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The taxonomic status of the common, stream-dwelling water strider, *Aquarius remigis* (Say) (Hemiptera:Gerridae) has been poorly understood. This species occurs throughout temperate and subtropical North America, and shows strong genetic and morphological variation across this broad geographic range. We present evidence from genetic, morphometric and breeding studies that populations previously identified as *A. remigis* in the southeastern USA constitute a new and separate species, *A. remigoides* (Gallant and Fairbairn). An analysis of genetic variation at 13 allozyme loci in 63 populations from across North America reveals significant genetic differentiation of populations in this region. Detailed analysis of a broad transect containing 43 populations from Georgia to Quebec reveals a narrow transition (hybrid) zone, with a genetic distance of 0.30 between the two species. UPGMA cluster analysis based on genetic distances among 11 species in the genera *Aquarius*, *Gerris*, and *Limnoporus*, reveals a greater genetic distance between *A. remigoides* and *A. remigis* than among any of the species in the other two genera. Significant deficiencies of heterozygotes and female-biased sex ratios in transition zone populations indicate strong barriers to gene flow. This is confirmed by laboratory crossing experiments which reveal severely reduced hatching success, reduced nymphal survivorship, and a strongly female-biased sex ratio in F1 hybrids. *A. remigis* and *A. remigoides* differ significantly in both size and shape, and can be reliably distinguished morphometrically through characteristics of the male genitalia. *A. remigoides* appears to be restricted to the eastern drainage of the Appalachian mountains, but further sampling is required to determine the western and southern range limits.

11-006

PHYLOGENETIC RELATIONSHIP OF JAPANESE MACROGERRIS SPECIES (HETEROPTERA: GERRIDAE), INFERRED FROM MITOCHONDRIAL DNA SEQUENCES.

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In spite of several excellent works on the phylogeny and taxonomy of the Gerridae, relationship among species in several genera and subgenera remains uncertain.

Phylogenetic relationship among species belonging to the subgenus *Macrogerris* of *Gerris* is difficult to analyse by external and other biological characters. In this paper, we inferred the phylogeny of Japanese water striders in this subgenus by analysis of nucleotide sequences of portions of two mitochondrial DNA regions (Cytochrome b and 16S ribosomal RNA).

Phylogenetic analysis showed that three Japanese *Macrogerris* species diverged into two clusters, i.e. *Gerris* (*M.*) *gracilicornis* and *G.* (*M.*) *yezoensis* are one group and *G.* (*M.*) *insularis* is the other. However, mtDNA sequences could not distinguish *G.* (*M.*) *yezoensis* from strains of *G.* (*M.*) *gracilicornis*. The analysis also showed that *G.* (*M.*) *insularis* diverged into two distinctly separated groups and that there is a relatively large evolutionary distance between them.

In order to see the evolutionary history of the subgenus *Macrogerris*, we also analysed genetic variability within and between *Macrogerris* species and compared with that of several species of other related genus and subgenus (*Gerris* and *Aquarius*).

11-005

PHALLIC PHYLOGENY - THE RELATIONSHIPS OF TREPOBATINE WATER STRIDERS (GERRIDAE) BASED ON DEEP GENITALIC STRUCTURES

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Water striders of the subfamily Trepobatinae are widely distributed in the New World, but have a more scattered distribution in the Old World, occurring only in Africa, southeast Asia, Melanesia and Australia. Our phylogenetic and zoogeographical analysis of this subfamily strongly indicates a Gondwanan origin, with subsequent radiation northward. Here we present the phylogeny of the tribe Trepobatini, which has one widespread genus in the Americas, and seven genera with much more restricted distributions in Australia and Melanesia. The morphology of the male genitalia show a linear pattern of modification along the zoogeographical progression of genera in western Melanesia, in particular a progressive reduction in the complement of endosomal selerites coupled with strong sclerotization and sculpturing of the phallosome, the latter a phenomenon unknown elsewhere in the Trepobatinae.

11-007

VARIATIONS IN RIBOSOMAL AND MITOCHONDRIAL DNA SEQUENCES AMONG SEMIAQUATIC BUGS (HETEROPTERA, GERROMORPHA).

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The infraorder Gerromorpha contains more than 1,000 semiaquatic bugs belonging to 8 families. Many groups of Gerromorpha are well known both ecologically and morphologically. Its higher classification and phylogeny are also well studied. However, little is known about genetic diversity and uniformity among gerromorphan families and genera. This paper deals with DNA base sequence variations in genomic ribosomal RNA and mitochondrial genes among gerromorphan families and genera. In this paper, we will discuss about the rate of evolution and phylogenetic application of DNA sequences.

Portions of two mitochondrial genes (16SrRNA and Cytochrome b) and a genomic DNA (28SrDNA) were amplified from individual insects by PCR and sequenced directly from PCR products. In total 27 species representing 14 commonly recognized genera of *Gerridae* and other related families (*Veliidae*, *Mesovelidae* and *Hydrometridae*) were examined.

The preliminary analysis showed that divergence in 28S rDNA sequences among species within *Veliidae* is very much higher than that of gerrid species. The phylogenetic tree generated by UPGMA method suggests that the genus *Microvelia* may be closely related to gerrid insects more than other veliid genera. This tendency was always observed in phylogenies derived from both ribosomal and mitochondrial DNA sequences. Now, we are expanding such analysis to other gerromorphan insect groups.

11-008

THE STATUS OF THE LARGER WATER STRIDERS IN DENMARK (HETEROPTERA, GERRIDAE)

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The three larger water strider species found in Denmark: *Limnoporus rufoscutellatus* (Latreille), *Aquarius najas* (De Geer), and *Aquarius paludum* (Fabricius), differ widely in habitat use, life history, and dispersal strategy. We presents the results of a survey of the past and present distribution and current status of these species in Denmark. Particular attention is paid to potential threats against the survival of these conspicuous insects in the Danish fauna.

11-009

MATE CHOICE AND PARENTAL DECISION-MAKING IN THE WATER BUG *BELOSTOMA FLUMINEUM* SAY (HETEROPTERA: BELOSTOMATIDAE)S.L. Kight, K.C. Kruse¹

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Female giant water bugs, *Belostoma flumineum* Say, oviposit their eggs upon the backs of male conspecifics. Males subsequently provide care for eggs until they hatch. Bearing eggs is costly to males in terms of increased predation risk, decreased foraging, and minimal polygynous mating. We therefore expect selection to favor males that are "choosy" in terms of mating and parental expenditure. Our findings support this prediction. Males actively terminate care to small egg pads if additional eggs are not received, or if the size of the egg pad is reduced shortly after oviposition. However, if reduction occurs after males have several days experience with eggs, they are unlikely to reject egg pads of any size, indicating that the "value" of eggs increases with age. Courting males prefer to mate with heavily gravid females, thereby reducing the likelihood of receiving a small complement of eggs. The number of eggs retained by a fully gravid female is proportional to the backspace of a male, and gravid females prefer males with no eggs already on their backs. Further study will address seasonal differences in male backspace availability and preference patterns of males that are partially encumbered and females that are partially gravid.

11-010

COMPARATIVE STUDY OF MATING SYSTEMS IN WATER STRIDERS : SEXUAL DIMORPHISM AND POST COPULATORY GUARDING

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The mating systems of water striders *Aquarius elongatus*, *A. paludum insularis*, *A. p. amamiensis* and *Gerris lacustris latiabdominis* in Japan were studied.

In *A. elongatus* and *A. p. amamiensis*, males established mating territories suggesting a system of resource defense polygyny. Males of *elongatus* have longer middle legs and *amamiensis* have more incrassate fore legs than females (sexual dimorphism). Non-contact guarding after copulation was seen in *elongatus*, while, males of *amamiensis* guarded females riding on their back after copulation (contact-guarding).

In *A. p. insularis* and *G. l. latiabdominis*, males were non-territorial suggesting a system of scramble competition polygyny. No marked sexual dimorphism in legs. Males of these species usually rode on females' back after copulation (contact-guarding). However the contact guarding was less developed in *G. l. latiabdominis*.

11-011

REVISION OF THE ORIENTAL HALOBATINAE GENUS *VENTIDIUS* DISTANT AND THE ZOOGEOGRAPHIC ANALYSIS INCLUDING ITS ALLIED ORIENTAL GROUPS (HEMIPTERA: HETEROPTERA: GERRIDAE)

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The Oriental water strider, genus *Ventidius* Distant (Gerridae: Halobatinae) is one of the habitants of river and the slow current of mountainous stream. Its taxonomical statue was somewhat confusing. This paper presents the taxonomical revision of all the 25 species including 5 new species. Based on its cladistic analysis, the species groups are provided. The difficulties of some taxonomical groups have drawn special attention, especially the *V. modulatus*-group. The phylogenetic reconstruction by the method of cladistic analysis will be also provided. The zoogeographic analysis of the genus and its allied genera in tribe Metrocorini will be discussed.

11-012

POPULATION BIOLOGY OF GIANT WATER BUGS IN ARGENTINA (HETEROPTERA, BELOSTOMATIDAE, BELOSTOMATINAE)

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Males of all Belostomatinae species brood eggs that are attached to their backs by conspecific females during the course of mating. This unusual reproductive behavior has made it possible to elucidate some relevant aspects of the population biology of several species from the Province of Buenos Aires for the last two decades. This long term research deals mainly with the most representative species in the study area, *Belostoma oxyurum* (Dufour).

Obtained results include quantitative data on sexual ratio and fecundity seasonal trends, and their effects on intrasexual competition, as well as on the relative and absolute shortage of the sexual resource represented by the male back space. These population traits provide basic information to speculate on which is the main social mating system these water bugs might exhibit.

Detailed information is included concerning the influence of density-dependent and density-independent factors on *B. oxyurum* and on its closest congeneric competitor, *B. elegans* (Mayr) in a permanent pond from the above referred area. Both species adaptive strategies are described based on the r and K demographic parameters.

Reproductive behavior of *B. oxyurum* based upon experimental studies carried out under laboratory conditions is described and include detailed observations of courtship, mating, egg-laying and brooding. These observations are compared to those recorded for North American Belostomatinae (*Abedus herberti* Hidalgo and *Belostoma flumineum* Say).

11-014

FITNESS BENEFITS OF PROLONGED MATE GUARDING IN *AQUARIUS CONFORMIS* (HEMIPTERA, GERRIDAE).

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During the mating season in the water strider *Aquarius conformis*, males clasp females continuously for many hours, mate occasionally with them, and guard them from takeovers during foraging, copulation and oviposition. Females lay eggs in batches on the underside of objects such as grass stems floating or fixed at the water surface, making egg counts convenient and accurate. Laboratory experiments showed, significantly, that females lay more eggs if fed more food, and that guarded females capture more food and lay more eggs unguarded females. Females thus increase their fitness by allowing males to clasp them for prolonged periods. Given the high likelihood of sperm precedence in this species, male fitness is concomitantly likely to be increased.

11-013

LIFE HISTORY DEPENDENT BEHAVIOURAL VARIATION IN WATER STRIDERS (*AQUARIUS REMIGIS*; HETEROPTERA: GERRIDAE)

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We investigated behavioural consequences of life history states in *Aquarius (Gerris) remigis*, a hemipteran surface predator and scavenger of small North American streams. A repeated-measures field study comparing reproductive (R) and non-reproductive (NR) first summer generation females (F) and males (M) during the same time in summer showed that NRM and NRF, which are foraging to survive the winter, behaved essentially alike, often being territorial. In contrast, RF and RM were more mobile than NR, RM being much more mobile than RF, always in search of and attacking potential mates. This resulted in RM showing little territoriality and having lower foraging success than all other categories, while RF had higher foraging success than NRF or NRM, thus likely increasing their fecundity. Many behavioural variables did not change with time. Exceptions were a decrease in the mating activity of R of both sexes, the number of mating attempts, mobility, and the stride rate of RM, and an increase in overall foraging activity of all individuals. This indicates that the behaviour of RM converged towards that of NRM as the season progressed. Individuals were assigned to either state post-hoc according to whether they had been seen mating at least once during the study period (July and August). Females were further tested to ascertain if they laid eggs. These results support the hypothesis that diapause and reproductive state to some degree predetermine behaviour, thus constraining behavioural flexibility. Attributing behavioural variation to various proximate causes can be central to the interpretation of alternative strategies and tactics.

11-015

WHERE THE TEXTBOOK MEETS THE POND: LIFE HISTORY VARIATION OF WATER STRIDERS IN NATURE

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Current theory on the evolution of life histories is based in part on *a priori* assumptions about the traits involved. For instance, most models assume that adult size is an increasing function of development time, and that size determines fecundity. These assumptions imply a trade-off, and an intermediate optimum can be found where the fecundity benefits of larger size are compensated by the cost of a prolonged development time. We tested these assumptions in a natural population of the water strider *Gerris buenoi* (Heteroptera: Gerridae).

In a large field experiment with individually reared bugs, repeated in the spring and summer generations over three years, we consistently found a negative correlation between development time and adult size instead of a positive one. The estimated genetic correlation was negative, and an additional experiment with different rearing conditions produced similar results as well. Variation in overall vigor dominates in our study population, rather than a trade-off between development time and adult size.

We examined if protozoan gut parasites, which have been shown to affect physiological condition of gerrids adversely, might be a cause of this variation in vigor. Only trypanosomatid flagellates showed significant effects on gerrid life history traits, but they were rare in our study population, and therefore cannot substantially influence population processes.

Several measures of female reproductive performance were uncorrelated with body size: lifetime fecundity, reproductive lifespan, the volume of eggs, and the proportion of eggs hatched. An experiment under food limitation did not show any benefits of larger size.

Despite our replicated and unusually large field experiments, we did not find the associations between life history traits commonly assumed by current theory. Our results, together with those of some earlier studies, challenge the generality of optimality models assuming particular trade-offs. We propose that the range of models in life history theory should be expanded to accommodate stochastic and neutral models.

11-016

SEASONAL DYNAMICS OF STAGE-STRUCTURED POPULATIONS OF THE WATERSTRIDER SPECIES *GERRIS LACUSTRIS* AND *GERRIS ASPER* (HEMIPTERA: GERRIDAE)

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The seasonal dynamics of *G. lacustris* and *G. asper* was studied in two markedly different habitats. The first (near Berne) is a typical habitat of *G. lacustris*, whereas the second (near Thun) is a cool shadowy pond, the only habitat type from which *G. asper* has been recorded on the Swiss Plateau. To sample waterstriders we used a square wooden frame (50 x 50 cm). 49 and 31 samples were taken every three or four days from early May to late October 1994. 24 and 20 sampling units per date were sampled on the two ponds. Species and stage of the captured waterstriders were determined in the field, if possible, and the sampled individuals were released immediately after counting. The sampling area in both populations was less than ca 3% of the total water surface.

The populations in the two habitats showed marked phenological differences. First instars of *G. lacustris* emerged in the second week of May in the population near Berne, and one month later near Thun. Emergence of summer adults near Thun was also delayed for one month compared with the Berne population where first summer adults emerged by the end of June. Despite this difference, *G. lacustris* was partially bivoltine in both locations. *G. asper* occurred only on the pond near Thun where it was univoltine. Maximum densities of all postembryonic stages of *G. asper* were much lower than those of *G. lacustris* in the same habitat. We used the index of dispersion to evaluate the spacing pattern of waterstrider nymphs and adults. As a rule *G. lacustris* showed a clumped spatial distribution. When densities were low, as was the case for most samples of *G. asper* and some of *G. lacustris*, the distribution pattern could not be discriminated from a Poisson distribution.

We used the Kiritani-Nakasuji-Manly (KNM) method to estimate stage specific survival rates, stage durations and numbers entering the six postembryonic stages. The KNM-estimates will be compared with those of another model and also with the results of a simulation approach. The reliability of the different estimators will then be discussed with special emphasis on the assumptions of the different methods.

11-017

UPS 'N DOWNS OF A 12-YEAR BUG COUNT: THE TALE OF TWO POND SKATERS

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Populations of adult waterstriders were studied by marking and weekly census on a small pond in northcentral Alberta, Canada. Individuals of 5 species colonized the pond shortly after ice-off in mid-April. Disappearance of adults from the surface varied more between years, occurring early-September to mid-October, and was associated with late spring conditions that affected the extent of a second generation. Of the 5 species of colonists, only *Gerris buenoi* and *Limnoporus dissortis* were regularly successful; their performance has been remarkably and positively correlated. In recent years populations of *G. comatus* have recruited more strongly although rates of colonization have not increased significantly for this species. Neither *G. pingreensis* nor *Aquarius remigis* have ever recruited significantly, despite regular colonization. These data suggest that associations between species and pond-type depend more on factors affecting recruitment than on those determining colonization. Study of yearly recruitment:colonization ratios and weekly records of the arrival and disappearance of bugs establish a pattern of large-scale colonization and departure over 8-10 week periods each spring. The research emphasizes the importance of flight for these waterstriders and shows that local populations are regularly linked by interpond movement both within and between breeding seasons.

11-018

MIGRATORY SYNDROME IN OVERWINTERING ADULTS OF TWO WATER STRIDERS, *AQUARIUS PALUDUM* (F.) AND *GERRIS LATIABDOMINIS* MIYAMOTO (HETEROPTERA: GERRIDAE).

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Most of overwintering macropterous adults histolyzed their mesothoracic indirect flight muscles before the middle of May in a wing dimorphic species *Aquarius paludum* (F.), whereas most adults of monomorphic (macropterous) species, *Gerris latiabdominis* MIYAMOTO kept their flight muscles and showed high flight propensity until late spring in Kochi, Japan. Macropterous adults of *A. paludum* had more mature eggs than those of *G. latiabdominis* in the middle of May. The loss of water surface and following starvation induced histolysis of the flight muscles and elongated surviving period in *A. paludum*, while adult *G. latiabdominis* under these conditions kept their flight muscles and survived for no longer period than the adults provided with sufficient foods and water surface. When macropterous adults fed on sufficient foods, loss of water surface made some adults of *A. paludum* stop laying eggs, although it can only reduce the number of eggs in *G. latiabdominis*.

If the loss of water surface occurs in the field, *A. paludum* may enter diapause and wait for rainfall and *G. latiabdominis* may move onto another water surface by flight.

11-019

DISPERSAL IN *ARCTOCORISA CARINATA* (SAHLB.) AND *CALLICORIXA PRODUCTA* (REUT.): THE EFFECT OF POPULATION DENSITY AND RESOURCE AVAILABILITY (HETEROPTERA: CORIXIDAE)V. I. Pajunen, I. Pajunen¹

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Dispersal of colour-marked *Arctocoris carinata* (Sahlb.) and *Callicorixa producta* (Reut.) adults in a rock pool environment was monitored for most of the reproductive season. The average daily dispersal was 10-30 %, *C. producta* being more active. Differences between experimental rock pools from which larvae had been removed and control pools were small. Only during short periods when resources in control pools were overused, more intense dispersal was observed.

The results suggest that in both species there exists a strong innate tendency to disperse, and local population density and food availability are factors of minor importance. Although intraquild predation lowers the reproduction success in rock pools with high larval density, preference for patches with low population density could not be demonstrated.

11-020

A LONG TERM MARK-RECAPTURE STUDY OF THE
COMMON EUROPEAN WATERSTRIDER
GERRIS LACUSTRIS (HEMIPTERA: GERRIDAE)

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The seasonal dynamics of a small population of the wing-polymorphic waterstrider *Gerris lacustris* was studied from 1986 to 1996 using the stochastic Jolly-Seber mark-recapture model. Adult gerrids were sampled weekly, and on their first capture they were marked individually with a simple code. Since the start of this study more than 12'000 individuals were marked. Sampling fraction was high (0.88 ± 0.095). Adult summer emergence ranged from 500 to 2000 individuals, and in eight out of ten years adult production was between 800 and 1400 individuals. The proportion of longwinged (LW) individuals was variable. In spring generations the proportion of LW ranged from 0.30 to 0.53, with a mean of 0.37 (summer: 0.37 to 0.53, mean: 0.44). The absolute number of both LW and shortwinged (SW) individuals of the summer generation was directly proportional to the number of LW and SW spring breeders with a proportionality factor of 4.5 ± 0.43 . The population was partially bivoltine, and the proportion of nondiapauses females was negatively related to the Julian date when the first 10% of the summer adults had been marked. The partial second generation accounted for less than 20% of the yearly production. Its absolute size was dependent on the number of nondiapauses females. Early start of adult moulting in summer, however, did not influence total adult production. LW and SW individuals differ in their observed residence times: LW individuals have shorter mean residence times (2 weeks) than SW individuals (3 weeks), and, furthermore, nondiapauses SW individuals show much longer mean residence times than diapauses SW individuals (6 weeks vs. 3 weeks). Overwinter mortality of SW adults varied from 0.64 to 0.92 (mean: 0.80). LW individuals do not as a rule hibernate in the pond area but disperse by flight in late summer / early fall, and their disappearance rates were correspondingly high (0.93 to 0.99, mean: 0.97). A simple model will be presented to partition the mortality of SW adults between first capture in summer and reappearance in spring into 1) mortality on the water surface and 2) mortality during hibernation on land.

11-022

MARINE INSECTS - INTRODUCTORY REMARKS
SYMPOSIUM ON ECOLOGY OF MARINE INSECTS

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Insects are the most abundant animals on land but relatively few species are known in marine environments. Although there are no known physiological barriers for a marine existence, evolutionary history coupled with special adaptations necessary for dealing with harsh physico-chemical conditions in the sea may be chiefly responsible for this paucity. However, at least 20 insect orders have marine species which spend one or more of their developmental stages in a marine habitat.

Most marine insects are wingless, flightless or weak fliers. Some 75% are in the orders Hemiptera, Homoptera, Coleoptera and Diptera. The most ubiquitous genus is perhaps *Halobates* (Hemiptera: Gerridae), the only known insect in the open ocean. The majority of *Halobates* species occur nearshore as do members of the Veliidae and Hermatobatidae (both Hemiptera). Marine Diptera include mosquitoes and biting-midges of economic importance, as well as beach flies and midges that may be mere nuisances. Both *Pontomyia* and *Clunio* (Diptera: Chironomidae) have truly submarine larvae and life cycles closely synchronised with the lunar or tidal rhythms. Most marine beetles occur in the intertidal but there are no truly marine families. The Chathamidae (Trichoptera), with 2 genera and 4 spp., are exclusively marine.

These are but a few examples of the variety of marine insects that one may encounter on beaches, salt marshes, mangrove swamps, coral reefs and the open sea.

11-021

ORIGINS OF EXTREME STRUCTURAL DIVERSITY IN THE
TWO BASAL CLADES OF THE HETEROPTERA

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The advanced Heteroptera are rather monotonous structurally while two basal clades, Enicocephalomorpha and Dipsocoromorpha (monophyletic ?) show great structural plasticity. It concerns the basic architecture of labium, pterothorax, forewings, coxal articulation, posttarsus, male abdomen and terminalia (sexual counter-trends: the more complex the male the simpler the female), adult evaporatoria and repugnatorial gland openings (tagmatic shifts from abdominal to modal thoracic position). This structural diversity does not fit the allegedly uniform biology (generalized predators) and habitat preferences (inhabitants of interstices in litter, moss and riparian gravel). The following explanations are possible: retention of the results of evolutionary experiments; results of strong sexual selection; adaptive consequences of an unidentified niche micropartitioning; unique SMR systems.

11-023

MOONLIGHT AND TIDAL CYCLES AS TIME CUES FOR
METHAMORPHOSIS AND EMERGENCE IN INTERTIDAL
MIDGES (DIPTERA: CHIRONOMIDAE)

D. Neumann

ABSTRACT NOT RECEIVED

11-024

ECOLOGY OF THE FLIGHTLESS MARINE MIDGE *PONTOMYIA* (DIPTERA: CHIRONOMIDAE)K.-F. Chen, C.-S. Chen¹ and K. Soong

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Semimonthly emergence cycles were found in *Pontomyia oceana* in south Taiwan. Each bout lasts for about 5 nights with peaks occurred at day 3 and 18 of a lunar cycle, when large numbers of adult males skiing on water surface with modified wings were attracted to light and hooked under-surface vermiform females for mating several hours after sun-set. A negative correlation was found between water temperature of the tide pools and the mean time of occurrence after sun-set from July to December 1992. In *P. natans* only males were found and they occurred every night, with fluctuations in numbers throughout the months. In the Laboratory, fertilized eggs of *P. oceana* were raised in coral fragments, and they hatched in 3-4 days under 25°C. Larvae build tubes made of small pieces of fragments, while first emergence occurred 22 days after hatching. Male *P. oceana* emerged earlier than females. The males remain active for about 2.4 hours, whereas females lay egg strings near surface in about 2.3 hrs. Modification of sun-set time and temperatures resulted in shift in timing of emergence of the day, but not the day after. It seems that sun-set time cues the starting time of the processes leading to eventual emergence, the rate of which controlled by temperature.

11-025

THE EFFECT OF TIDAL VARIATION ON 'GOOD GENES' SEXUAL SELECTION IN SEAWEED FLIES, *COELOPA FRIGIDA* (DIPTERA: COELOPIDAE)

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Female seaweed flies (*Coelopa frigida*) prefer to mate with large males. Mate preferences are thought to have evolved in response to several types of sexual selection: the fitness of offspring is increased as a result of female choice both by the production of attractive sons (the Fisher process), and by the production of offspring with greater survival ('good genes'). There may also be direct benefits to discriminating females in terms of increased longevity. The contribution of good genes sexual selection was estimated by determining the correlation between the pattern of willingness to mate and the predicted mean progeny fitness of each type of male. Mating patterns were measured in pairwise laboratory trials, and offspring fitness by estimating the deviation from Hardy-Weinberg proportions of a large inversion karyotype. A significant relationship was found in samples from British populations, but not in samples from Sweden. It is suggested that the strength of good genes selection depends on ecological factors associated with the tidal variation to which natural populations are exposed. The effect of tides on the evolution of mating behaviour is discussed.

11-026

MARINE CADDISFLIES (TRICHOPTERA: CHATAMIDAE)

J. Leader (Dunedin-New Zealand)

11-027

ADAPTATIONS OF *CAFIUS* (COLEOPTERA: STAPHYLINIDAE) TO ITS SEASHORE HABITAT

J.H. Frank (Gamesville-United States)

ABSTRACT NOT RECEIVED

ABSTRACT NOT RECEIVED

11-028

THE CHEMICAL ATTRACTANTS OF MALE *TROCHOPUS* (HEMIPTERA: VELIIDAE): A PRELIMINARY REPORT
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Although marine insect are rarely encountered, several species are frequently found in high densities around tropical island shores. *Trochopus* is a neotropical genus with five known species, confined to central and south American coasts and occupies habitats similar to those of the much better known sea-skaters *Halobates*. During a recent expedition to the Bahamas Islands the small veliid *Trochopus plumbeus* (Uhler) was found in considerable densities in all visited islands.

Large aggregates, with several hundred specimens, encountered at Joe Sound Long Island allowed us to collect significant numbers of that marine insect for field bioassays and laboratory experiments. Adult specimens were sexed and males and females were kept in separate containers. From the field trials it was quite clear that females were attracted to males as well as to male extracts but not the opposite.
Gas Chromatography-Mass Spectrometry analyses performed on the extracts of both males and females revealed the presence of a series of aldehydes and long chain aliphatic acids that are water insoluble and could possibly act as surface-dispersible semiochemicals. The differences in the chemical composition of the two sexes and their potential role in the insect's chemical communication system will be discussed.

11-030

ECOLOGICAL PHYLOGENETICS AND HISTORICAL BIOGEOGRAPHY OF SEA SKATERS, *HALOBATES* (HEMIPTERA, GERRIDAE)

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The oceanic *Halobates* are remarkable insects with a unique way of life and there has been much speculation about their origin and evolution. The recent discovery of a freshwater relative to sea skaters, *Austrobates*, adds an exciting new piece to the jig-saw puzzle picturing the evolution and diversification of marine water striders. It is suggested that ancestral Halobatini lived in both limnic and marine habitats and that *Austrobates* and *Halobates* evolved from their euryhaline ancestors into species preferring only limnic and marine habitats, respectively. The single known species of *Austrobates* is endemic to Cape York Peninsula while the subgenus *Hilliella*, the sister group of all other *Halobates* species, is distributed along the coasts of tropical northern Australia. It is therefore hypothesized that the sea skaters evolved somewhere in the area which now constitutes the northernmost part of the Australian continent. Fossil suggests that *Halobates* had evolved before the Middle Eocene (45 Mya), when Australia was part of eastern Gondwanaland. The known distribution of *Halobates* covers the whole Indo-Pacific region (only one species has invaded the Atlantic Ocean) and there are endemic, coastal species in such distantly separated places as the Red Sea and the Galapagos Islands.

11-029

REEF-TREADERS: OBSERVATIONS ON THE BIOLOGY OF AN EXCLUSIVELY MARINE INSECT FAMILY (HEMIPTERA: HERMATOBATES).

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The reef-treaders (Hermatobatidae) are an exclusively marine family of insects that occupies a somewhat isolated phylogenetic position within the semi-aquatic bugs (Gerromorpha). Observations in the south-west Pacific show that they can be abundant on shores that provide rocks with cavities that remain air-filled at high tide. The vertical zonation of cavities suitable for the insects is restricted to mid-to-lower regions of the intertidal zone. The behaviour of the insects during the tidal cycle is described. The adults appear to swim further from the shore than do the larvae, and they were observed copulating in tidal pools. The insects are clothed in a layer of micro-hairs of unique design: a short tapering shaft tipped with a sphere. These hairs may act as a plastron: their distribution corresponds precisely to the pattern of the silvery air-layer seen when the insects are submerged.

11-031

HOW OCEANIC SURFACE ENVIRONMENTS AFFECT THE DISTRIBUTION OF PELAGIC *HALOBATES* (HEMIPTERA: GERRIDAE)?

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Only five species of the ocean skater *Halobates* have successfully colonized the open ocean. They occupy a vast area extending from latitudes 40°N to 40°S of the equator. They are totally wingless and live in a two-dimensional world. Confined to the ocean surface they are exposed to various physical forces acting at the sea-air interface. Oceanic diffusion, which acts constantly to disperse them, must be one of the important factors responsible for the distribution and life history of *Halobates*. We estimated its effects on the following aspects of *Halobates* by using oceanic diffusion diagrams constructed by Okubo (1971, 1974), and the mathematical model developed by Kierstead and Slobodkin (1953) and Skellam (1951):-
1) Oceanic diffusion can carry ocean skaters from an initial patch as far as 2,500 km apart in 60 days. This distance is equivalent to ca. one-sixth of the maximum width of the Pacific Ocean where *H. micans* is found.
2) Mutual encounter rates as facilitated by oceanic diffusion could be high (1.5/day) even at very low population densities (1/km²). This suggests that a *Halobates* could find a mate even when an individual had been carried a long distance away. Thus, extensive gene mixing could occur over the whole range of a species' distribution.
3) We calculated the growth rates of *Halobates* as a function of distribution range and diffusivity. The estimated growth rates are rather low (0.0026-0.0079/day) compared with those of other insects. However, they are offset by a relatively long life span and an extended oviposition period (perhaps over 2 months).
Thus, pelagic *Halobates* appears to have adopted a strategy of slow growth and prolonged longevity to cope with living in an unstable and harsh physical environment.

11-032

MITOCHONDRIAL DNA AND *HALOBATES* (HEMIPTERA: GERPIDAE): TECHNIQUES FOR TRACKING TINY TRANS-OCEAN TRAVELERS.A. M. Shedlock, F. A. H. Sperling¹ and L. Cheng²

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Of the 45 described species in the genus *Halobates*, 5 have evolved a remarkable suite of adaptations and are fully pelagic, found mostly on tropical oceans between latitudes 40 degrees N and S. Each species exhibits a distinct zoogeographic pattern. *H. micans* is cosmopolitan and found in all three oceans; it is the only species found in the Atlantic. *H. sericeus* is amphitropical, the northern and southern populations being separated around the Equator by *H. micans*. The other three species are more coastal in habitat: *H. germanus* occurs in the Indian Ocean and the western Pacific while *H. sobrinus* and *H. splendens* are found off the coasts of central and south America.

These fascinating and enigmatic insects occupy one of the largest continuous ecosystems on the Earth and challenge our conventional wisdom about adaptations and speciation in the pelagic marine environment. To help understand the phylogeographic nature of this system and to complement morphological and behavioral investigations, we have initiated a comprehensive molecular genetic study of *Halobates* using PCR, DNA fingerprinting and nucleotide sequence analysis. We present preliminary data on inter- and intra-specific variation of the mtDNA cytochrome oxidase II sequence of several *Halobates* species and outgroup taxa. Results will be discussed within the context of both conventional phylogeny among species as well as prospects for large-scale studies of gene flow in pelagic populations.

11-034

AUSTRALIAN WOOD-MINING CHIRONOMIDS

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In 1988, after a postdoctoral year in Canada, I felt able to join Don Oliver in commenting on northern hemisphere aquatic wood-mining chironomids (Cranston and Oliver, 1988). After eight years of study of the Australian chironomid fauna, a substantial wood-mining chironomid fauna, potentially even more diverse than that of the northern hemisphere has been revealed. Some taxa have been recovered and reared from immersed wood, others are recognized by the wood fibers in the gut, combined with some distinctive head capsule morphologies.

For the Holarctic fauna, Don Oliver and I observed that wood-miners are a paraphyletic assemblage, and we speculated on the convergence of mentum morphology amongst the miners belonging to the subfamilies Orthocladiinae and Chironominae. The menta of a variety of Australian wood-miners will be illustrated, showing that these observations are true also for many Australian wood-mining taxa.

Pupal exuvial evidence suggests that among the Chironomini only *Stenochironomus*, *Harrisius* and the recently-described *Imparipecten* are all common. Amongst the Orthocladiinae, *Austrobrillia* is frequent, whereas others, many of which are *Bryophaeocladus*-like, are at most sporadic in occurrence.

11-033

MORPHOLOGICAL ADAPTATIONS OF ADULT TROPICAL CHIRONOMIDS

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Chironomid wing length was used by McLachlan (1985) to describe habitat characteristics. He suggested a negative relationship between wing length of most abundant species and duration or predictability of its habitat.

Tropical chironomids are smaller or of the same size as their closest relatives in temperate areas. Likewise, the anal lobe and the number of setae on squama often are reduced, the VR often higher, the costal extension often longer, the antennal ratio often lower, and the leg ratio often higher, etc. Not all of these changes appear to take place in all species and certainly not always concurrently.

Several of the characters in which changes take place are important in keys and phylogenies, and it is necessary to take the climatic conditions of where the species were collected into consideration when judging whether a character shows homoplasy or not. In order to judge whether a character is caused by adaptation to a tropical climate or a tropical rain forest, it is necessary to compare a species with its closest relative or relatives from more temperate areas.

Certainly the habitat predictability of semiaquatic chironomids is high in a tropical rain forest with available habitats permanently present. When comparing species within a monophyletic group McLachlan's theory thus appears to hold.

11-035

THE CHIRONOMID SPECIES OF THE ITALIAN LAKES: A REVIEW

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Many large and important lakes are present in Italy, especially in the northern areas. These lakes are rich in chironomid fauna. Despite intensive research on zoobenthos of some of these lakes in the past, a complete list of chironomid species in any of these lakes is still lacking. In most cases, a list of genera or species groups was published. For example, in Maggiore lake, 72 chironomid taxa were captured, 29 were identified as genera, 17 as species group, and only 26 as species based generally on larval identification (Nocentini, 1989) and not adults. Such numbers of species identified is low when compared with the ones observed in some other lakes. For example, 184 species were identified in the Bodensee lake (Reiss, 1968). A review of the knowledge of chironomids in Italian lakes is now given. High organic discharges in some large lakes, such as Maggiore, Como, Garda, have necessitated lake restoration which is either planned or being implemented in some cases. This may cause a change in chironomid species composition. Therefore, it is necessary to know the present state and baseline information in order to be able to detect changes in species composition in the future. The collected specimens deposited in the Department of Biology, Section Ecology, University of Milan, have been re-examined and the complete list of species is compiled. Starting at the beginning of 1995, samples of adults were collected with a week or 15-day frequency in Maggiore lake (Angera), Varese lake (Schiranna), Como (Onno), Monate and Comabbio lakes. The information gathered will be compared with the existing information from the past and final conclusions about the trophic state of the lakes, as evidenced by chironomid fauna will be presented.

11-036

TROPHIC INTERACTIONS IN MIRE BENTHIC COMMUNITIES DOMINATED BY CHIRONOMID LARVAE

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Chironomid larvae dominated the zoobenthos in springs, streams and pools of Miyatoko Mire, Japan. A total of 32 species of chironomid adults were collected from the mire by rearing larvae, net-sweeping and light trapping. In a spring-fed pool, a detritivorous [*Stictochironomus akizukii* (Tokunaga)] and a carnivorous [*Procladius culiciformis* (L.)] midge were predominant. Both species were semivoltine with overlapping two cohorts emerging during May-August. Since the day-degrees for *S. akizukii* were adequate to complete its development within a year, some nutritional defects might have existed for this species. Annual production of *S. akizukii* larvae ($4.36 \text{ g m}^{-2} \text{ year}^{-1}$) was equivalent to those reported for large-sized chironomids in eutrophic waters. Production of *P. culiciformis* ($2.06 \text{ g m}^{-2} \text{ year}^{-1}$) also ranked high among the values reported for Tanypodinae larvae. Larvae of *S. akizukii* fed on small-sized diatoms and detritus while 3rd and 4th instars of *P. culiciformis* fed on first 3 instars of *S. akizukii* as well as on its own 1st and 2nd instars. Large-sized diatoms and desmids maintain high production of *P. culiciformis* while early-instar larvae of *S. akizukii* seemed to stimulate its larval growth before emergence.

11-038

WHY PROFUNDAL CHIRONOMIDS AND OLIGOCHAETES CAN BE USED AS INDICATOR ORGANISMS IN MONITORING LAKE TROPHY

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The trophic lake type system established at the beginning of this century has been fruitful in producing discussions of fundamental causal relationships between different aspects of limnology. Chironomids and later oligochaetes proved to be useful as indicator organisms in classifying lakes. The factors determining the faunal assemblages in the profundal zone of regularly stratified lakes are an interaction primarily between the quality and quantity of food and oxygen conditions. The available food originates exclusively from precipitating organic matter produced in the photic zone, except in relatively shallow oligotrophic lakes, which may also have a profundal production of phytobenthos. With increasing lake trophy the amount of food increases while oxygen concentration decreases.

Based on our own research and the literature review, we describe available food quality/quantity, feeding behavior, respiratory adaptations to oxygen depletion, and anoxic survival for a number of chironomids and oligochaetes characteristic for habitats with different trophic state and oxygen regime.

11-037

DROUGHT TOLERANCE OF LARVAE OF *CHIRONOMUS KIIENSIS* TOKUNAGA (DIPTERA: CHIRONOMIDAE) IN THE LABORATORY.

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Larvae of *Chironomus kiiensis* Tokunaga, one of the most dominant species in paddy fields of the Yamaguchi area, Japan, were reared under different drought conditions in the laboratory to determine the effects of drought condition in paddy fields.

After making nests, 2nd, 3rd and 4th instar larvae were kept in the substrata of which water content (W.C.) was 40%. Percentage survival of each instar decreased as drought period and all larvae died within 24-30 days. Second and 3rd instar larvae were more resistant to drought condition than 4th instar larvae in the mean survival percentage. Mature larvae, however, recovered better than younger larvae in percentage emergence when reared at 40% W.C. for 7 days.

Adults derived from 4th instar larvae experienced different drought periods (0-10 days) at 40% W.C. showed effects of drought condition. Percentage emergence distinctly decreased as drought period, and it increased as ages of larvae. Number of ovarian eggs showed no difference until 7 days, but decreased when it lasted 10 days. In conclusion, larvae of *C. kiiensis* suffers from drought condition, but its population in paddy fields is supposedly able to continue when the period not longer than 5 days.

11-039

ORDINATION AND CLASSIFICATION OF SUBFOSSIL CHIRONOMID COMMUNITIES IN DANISH LAKES

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Parametric and nonparametric multivariate methods were used to classify and characterize the variation in subfossil chironomid communities in a wide range of Danish lakes. Canonical correspondence analysis was used to examine the relationship between chironomid assemblages and contemporary water chemistry with special emphasis on concentrations of total phosphorus (TP). A transfer function to infer lake trophic status (TP range 18-1,200 $\mu\text{g P l}^{-1}$) from subfossil chironomid remains was established using weighted averaging (WA) regression and calibration techniques.

11-040

INFESTATION PROBLEMS AND NEW STRATEGIES FOR CONTROL OF *PARATANYTARSUS GRIMMII* (SCHNEIDER). A CHIRONOMID MIDGE (DIPTERA: CHIRONOMIDAE) INFESTING ENCLOSED DRINKING WATER SYSTEMS IN THE U.S.A.

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In 1990, a small midwestern USA town water utility began receiving complaints of small worm-like organisms appearing in the municipal drinking water supply. These organisms were identified as the chironomid, *Paratanytarsus grimmii* (Schneider). In this particular system, the town's water is derived from groundwater sources and is pumped directly into a treatment facility. Upon entering the treatment facility, the water is pumped to an elevated storage tower for eventual distribution to consumers. The original midge infestation occurred through a vent in the water tower. Midge population densities circulating in the distribution system ranged from <1 to 40 individuals/100 liters of water. The larvae appeared to feed on dead bacteria from the water column. Because *P. grimmii* is parthenogenetic, it was able to reproduce in the enclosed distribution system. Two chemicals that are currently used in water purification and clarification were tested as control agents: Cat-Floc LS, a water clarifier, and Hydrogen Peroxide (H₂O₂), a water purifier. Both products caused mortality in larvae of *P. grimmii*, however, Cat-Floc was the most effective with a longer application time. The limitations and constraints of these products will be discussed.

11-041

CHIRONOMIDAE CONTROL IN A DRINKING WATER SUPPLY SYSTEM IN ISRAEL

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In 1992, chironomid infestations in a drinking water supply system in Tel Aviv were discovered. Continuous monitoring (1992-95) revealed that a majority of the covered storage tanks receiving Lake Kinneret water through the Israel National Water Carrier (INWC) was infested with all life stages of chironomids. Tanks draining and cleaning, sprinkler spraying water in the tank space, midge adult electrocution (traps) and the routine water chlorination (1 ppm) were inadequate measures for the midge control. Laboratory bioassays of 4th-instar midge larvae with monochloramine resulted in 24-h LC₅₀ and LC₉₀ values of 10.4 and 18.0 ppm, respectively. Addition of ammonium sulfate followed by sodium hypochlorite solution for "shock chloramination" of midges in a tank drained down to contain 20 cm deep water and then disconnected, was tested. All dead chironomids were flushed out through the drainage outlet at 5 h post-treatment. The tank was resupplied with water level raised to the operational volume while confirming its chloramine concentration within the legal limit. A second identical treatment of water in the tank is suggested 8-10 days after the first one to kill the midges hatched from eggs, and the adults, surviving the first treatment. Seven commercial treatments were made in 5,000 - 8,000 m³ storage tanks. Pre-treatment larval density at the tank bottom during the first treatment (August 1994) was 8,500 larvae/m², and 17,500 adults/light trap/night. The treatment eliminated all midge larvae and only 23 adults/light trap were taken after 5 days post-treatment.

11-042

INVASION OF ADULT CHIRONOMIDAE IN VENICE, ITALY: ENVIRONMENTAL, CLINICAL AND PREVENTIVE ASPECTS

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In the past decade, the city of Venice was "invaded" by dense swarms of adult Chironomidae, causing a variety of nuisance, economic and medical problems. Car, train and boat traffics to and from, and within, the city were severely affected. In August 1985, the safety of airplane traffic and the airplanes at the Marco Polo Airport near Venice were threatened due to massive accumulations of dead midges on the runways as well as entry of adult midges into delicate equipment on the aircraft. Continuing research on Chironomidae since 1983 has revealed that *Chironomus salinarius* inhabiting the lagoon of Venice is the nuisance midge, with larval densities ranging from 1,000 to 10,000/m². On the basis of midge larval and adult data, control has been attempted taking an integrated approach. Chironomidae are considered as environmental and occupational sensitizers contributing to respiratory diseases. The human sensitivity to chironomid allergens, particularly peptides complexed with hemoglobin(s) sub-units may induce asthma and rhinitis. Clinical and epidemiological screening to establish chironomid-related allergies showed that the prevalence of respiratory allergies was low in the general population, but nearly 5% of symptomatic children tested (skin test) with extracts of *C. salinarius* larvae and adults were positive. Among these children, 6 developed bronchial asthma and the rest rhinitis. Atopy plays a relevant role as predisposing factor in the sensitization. Chironomid allergies, at present, are not in the epidemic stage in Venice. However, continuing studies on chironomids and screening new cases of allergic sensitization in the Venice area are important.

11-043

CHIRONOMIDAE (DIPTERA) NUISANCE IN SOME WETLANDS LOCATED NEAR CAGLIARI AND ORISTANO, SARDINIA, ITALY

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Chironomid nuisance of great magnitude was recently noticed at first in urban areas of Cagliari and then in the cities of Oristano and S. Giusta, Sardinia, Italy. The Environmental Protection Department of Sardinia Region and the Public Health Office of ULSS 11 Venezia, jointly initiated research in May 1993 to determine the nuisance chironomid taxa and their larval distribution. It was also planned to collect data on selected chemical and physical parameters (e.g., depth, transparency, temperature, pH, Eh, salinity, and dissolved oxygen in the waters: and temperature pH, Eh, and % organic matter in the sediments) of the wetlands studied. In the framework of this research, 10 wetlands with different levels of salinity (>60‰ than freshwater) were studied. *Baeotendipes noctivaga* (K.) was the only chironomid species collected in a salt-farm near Cagliari, with mean density higher than 10,000 larva/m². *Chironomus* gr. *salinarius* was mainly present in saline waters near Oristano, while *C* gr. *plumosus* was abundant in freshwater areas

11-044

SIGNIFICANT RECENT ADVANCES IN THE CONTROL OF NUISANCE CHIRONOMIDAE (DIPTERA)

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In recent years, massive swarms of adult Chironomidae emanating from a variety of natural and artificial habitats distributed worldwide have been reported. These swarms have increasingly posed nuisance and economic problems and in some situations medical problems to waterfront residents, workers, visitors and business and industrial establishments. Among the many organophosphate (OP) insecticides used as midge larvicides, temephos has been the larvicide of choice in many situations. The insect growth regulators (IGRs), methoprene, pyriproxyfen and diflubenzuron are excellent substitutions, especially against temephos- or OP-resistant chironomids. Chemical formulation research has revealed the usefulness of sustained-release formulations of OPs and IGRs in providing satisfactory and more economical immediate as well as long-term control of chironomids. The bacterium, *Bacillus thuringiensis* serovar. *israelensis*, when used at high rates, is effective against some Chironominae. Some species of fish have reduced larval Chironomidae for short durations. Attraction of some adult Chironomidae to light (quantity or quality) can be useful to divert or trap the adults. Physical and chemical ecological changes of chironomid larval environments may result in reducing midge nuisance in some situations.

11-046

LIFE HISTORY OF
CALOPTERYX HAEMORRHOIDALIS
(ODONATA: CALOPTERYGIDAE).

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C. haemorrhoidalis lives in Southern Europe. Mating behaviour with threatening- and courtship flight (filmed in slow motion up to 500 frames/ second), and copulation as well as a computer animation of the internal processes (sperm removal) are presented. The film shows submerged oviposition, a computer animation of fertilization of the eggs, and the hatching of larvae from eggs. In summer a high density of individuals and high air temperatures are stress factors. How do they influence the population numbers of *C. haemorrhoidalis*? The film shows the results of our working group as well as other ecological aspects of this wide-spread mediterranean species.

11-045

STRATEGIES TO REDUCE THE ENVIRONMENTAL IMPACT OF CURRENT CHIRONOMID MANAGEMENT PRACTICES IN AUSTRALIAN RICE FIELDS

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Chironomid larvae, particularly those of *Chironomus tepperi* Skuse, are important pests of rice in New South Wales, Australia. They are effectively controlled with the organophosphorus insecticides malathion and chlorpyrifos, however, these compounds are currently under close scrutiny by the NSW Environment Protection Agency and other groups concerned with pesticide contamination in drainage water. The allowable drainage limit for chlorpyrifos is set at 1 ng/L, a level which will preclude the continued use of this compound as a larvicide.

Research is currently being directed towards identifying replacement compounds with a narrower spectrum of activity against aquatic organisms. Of those examined, the phenyl pyrazole compound fipronil is particularly promising. Development of rice varieties resistant to chironomid attack represents a possible method of reducing chemical inputs, and screening trials have shown that lines derived from the water weevil resistant variety WC-1403 may be particularly valuable. *C. tepperi*, which has only a single generation in rice fields, strongly avoids ovipositing into water conditioned by the presence of chironomid larvae, conspecific or otherwise. Identification of the semiochemical responsible may lead to the development of an oviposition deterrent that will allow further reductions in the use of synthetic insecticides.

11-047

FEEDING MECHANISM AND PATTERNS OF ADVANCED INSTAR LARVAE OF *UROTHEMIS ASSIGNATA* (SELYS) (ODONATA: LIBELLULIDAE)

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Under laboratory condition, 9th 10th and 11th instar larvae of *Urothemis assignata* (Selys) kept still on detecting prey, waiting for it to encroach on the strike range before it was captured. Stalking of prey was not common and grasping of prey was efficient, especially with small-sized prey. Ingestion followed successful capture of prey, interspaced by a brief period of rest.

The compound eye was the main organ for detecting the presence of prey while antennae and tarsi served as auxilliary organs. The movement pattern of prey significantly affected the ability of larva to detect and capture prey (ANOVA, $P < 0.05$). Larvae exhibited increased food intake as deprivation time increased, and got satiated at 48.88 min.

11-048

ODONATA IN TROPICAL FORESTS: DO THEY INDICATE THE CONSERVATION VALUE? THE ANALYSIS OF THE ODONATA-FAUNA IN FORESTS OF EASTERN IVORY COAST:

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Seven „Forêts classées“ in the Eastern Ivory Coast, Westafrica, are the subject of an extensive rehabilitation programme from the GTZ (Gesellschaft für Technische Zusammenarbeit, Eschborn, German ODA). The forests are in different state of degradation. To ensure a rehabilitation „réserves biologiques“ are excluded from further management. In Europe the use of the Odonata group in the implementation of conservation goals is quite common. There are no experiences in tropical countries.

We compared the Odonata fauna between different forest types and between small woodland rivers and open streams. Although the fauna of Odonata in the Ivory Coast has been already studied, serious problems of determination of the adults arised. We could identify at least 50 species, about 160 species are expectet in the Ivory Coast. The species can be classified to species of stagnant and flowing waters. Most of the year the forest rivers have running water. In the dry season the small rivers are divided in many small ponds for a period of about three months. The Odonata of running water bridge this period as adults. The point diversity of the Odonata fauna is high due to the diverse natural conditions in the tropical forest. The Odonata-community in the forest differs strongly from the species assemblage of the open stream. The quality of rivers is influenced by the whole watershed. A lot of species of Odonata are confined to primeval conditions so that their occurence indicates river- and forest-quality of high conservation value. In that way the study of Odonata is a useful tool for the evaluation of a very complex ecosystem.

11-050

COMPARATIVE MORPHOLOGY OF THE SECONDARY MALE GENITALIA OF SELECTED LIBELLULOID DRAGONFLIES (ODONATA: LIBELLULOIDEA)

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Internal and external cuticular morphology and musculature of the secondary genitalia, i.e., the penis and associated structures on the 2nd and 3rd abdominal segments, are described in male *Zoraena diastatops* Selys (Cordulegastridae), *Gomphomacromia paradoxa* Brauer (Gomphomacromiidae), *Oxygastra curtisii* (Dale) (Corduliidae*), *Didymops transversa* (Say) (Corduliidae*), *Cordulia shurtleffi* Scudder (Corduliidae*), *Macrodiplax balteata* (Hagen) (Libellulidae), and *Libellula incesta* Hagen (Libellulidae). *Zoraena* and *Gomphomacromia* retain symplesiomorphic penile morphology (4th segment symmetrical, sperm pump emptied upon compression of the sperm vesicle), while *Oxygastra*, *Didymops*, and *Cordulia* exhibit distinct torsion of the 4th penis segment and a sperm pump that fills upon vesicle compression; the Libellulidae s.s. have symmetrical penes (possibly apomorphically so) and a sperm pump that fills on compression. *Zoraena*, *Oxygastra*, and *Didymops* all have a well developed anterior hamular depressor muscle (M9a_{II}), whereas this is lost independently in *Gomphomacromia* and the libellulids. Libellulidae share the unique apomorphies of M9b_{II} being lost and M4_{II} and M6_{II} (both well developed) originating near the anterior margin of segment 2 rather than near midlength.

*Conventional family designations are used for convenience, although recent evidence suggests that the Corduliidae, even with Gomphomacromiidae s.s. excluded, are paraphyletic.

11-049

THE WARMING UP STRATEGY OF IMMATURE *AESHNA MIXTA* (ANISOPTERA: AESHNIDAE)

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In dragonflies, as in most other flying insects, the thoracic temperature has to reach a certain level before flight is possible. Dragonflies can warm up both ectothermically or endothermically (by shivering). This has already been shown in adults of the genus *Aeshna* and is thought to be absent in immatures. Because shivering increases the thoracic temperature, it is expected that this also reduces the duration of the pre-flight period. Since the rate of increase in thoracic temperature is related to ambient temperature, we also expected a negative relation between shivering and the ambient temperature.

We tested the presence of endothermic warming up and the effects of ambient temperature and shivering on the duration of the pre-flight period on 27 immature *Aeshna mixta*.

We show that also maturing *A. mixta* can use shivering. The duration of shivering was negatively, but just not significantly, correlated with the ambient temperature. The duration of shivering and the ambient temperature did not affect the duration of the pre-flight period. Shivering intensity however significantly reduced the time prior to take-off. Our results further suggest that abdominal shivering may be part of an endothermic strategy. We discuss our findings in the light of eocrepuscular flight, that until now has only been observed in mature dragonflies.

11-051

INTRASPECIFIC INTERACTIONS OF LARVAL ODNATES G.I. Ryazanova

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The spatial behaviour of dragonfly larvae was studied in the laboratory. Methods of direct observations and filming and video were used. Only significant quantitative results were discussed. The subjects were F - F-3 larvae of 14 zygopteran and anisopteran species.

Territorial competition has been found to characterize all the studied species. In middle and older instars territorial competition is not associated with person's age and sex. It is an adaptive species-specific and plastic behaviour. That territorial competition is one of the mechanisms for spatial organization of larval communities, regulating their distribution. It is determined by relative population density. That form of behaviour can be substituted by another form (aggregation or accidental distribution) in stress condition.

Cannibalism and the deficit of the refuges was been the factors of evolution of dragonfly larvae territorial competition.

The special form of territorial competition is shown by larvae of the last instar several days before emergence. That form is associated with the larval sex. That behaviour can be regarded as a precursor of the imaginal one in these hemimetabolic insects.

The investigation demonstrated the sample of the intraspecific interactions of watching predatory insects as exemplified by dragonflies.

11-052

THE MATING SYSTEM OF THE DAMSELFLY *LESTES SPONSA* (ZYGOPTERA: LESTIDAE)

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We studied a population of the damselfly *Lestes sponsa* which shows extreme contact mate guarding at a small shallow pond from 17 July till 11 August 1994. Every animal in the population was individually marked with a number written on its wings with a permanent marker, which made it possible to recognize animals without capturing them. Each day we walked around the pond every half hour recording every animal with its code, sex, position at the pond and reproductive state (solitary, tandem, copula or ovipositing). Males did not defend territories, instead there was intense interference competition with tandem pairs. We discuss how the adaptation of the genus *Lestes* to live in temporary ponds may have impeded the evolution of territoriality. Mate choice is thought to be very limited. Species recognition by males is reduced and thought to be unadaptive in this species. The mating system can best be described as 'female control' with encounter-limited mating by the males. This is the first description of a combination of two mating strategies sensu Conrad and Pritchard (1992) in one species. We discuss the predictions made by Conrad and Pritchard for the female-control mating system and make predictions about the occurrence of the combined mating type.

11-054

TAXONOMIC REVISION AND PHYLOGENETIC ANALYSIS OF THE GENUS *OREODYTES* SEIDLITZ (COLEOPTERA: DYTISCIDAE: HYDROPORINAE) BASED ON LARVAL MORPHOLOGY.

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The larval morphology of the dytiscid genus *Oreodytes* Seidlitz is investigated through a detailed analysis of 16 Nearctic and two Palearctic species. Many structural features, especially those of chaetotaxy and porotaxy of head capsule, head appendages, legs, last abdominal segment, and urogomphi were found to be useful for taxonomic and phylogenetic comparisons. Within the tribe Hydroporini, it is proposed that *Oreodytes* shares a monophyletic origin with the genera *Deronectes* Sharp, *Scarodytes* Gozis, *Nebrioporus* Régimbart, and *Stictotarsus* Zimmermann. The classification of members of *Oreodytes* indicated by larvae reveals to be only partly reconcilable with the one indicated by adults. Once subdivided into three species-group, *Oreodytes* is now comprised of four species-groups: the *O. picturatus*, the *O. obesus*, the *O. scitulus*, and the *O. quadrimaculatus* species-groups. *Oreodytes quadrimaculatus* was found to be more closely related phylogenetically to members of the *O. picturatus* and *O. scitulus* species-group which share a monophyletic origin. The genus-name *Neonectes* J. Balfour-Browne is suggested as a junior synonym of *Oreodytes* as it is hypothesized that their members are closely related phylogenetically with those of the *O. scitulus* species-group.

11-053

PHREATOBIONTIC BEETLES OF JAPAN (COLEOPTERA: PHREATODYTIDAE AND DYTISCIDAE)

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Ten species with a subspecies of phreatobiontic beetles are now known in Japan, all from the western parts of the main islands, though only three of them were previously described. They are: 6 species of *Phreatodytes* of the Phreatodytidae, 3 species and 1 subspecies of *Morimotoa* of the dytiscid tribe Hydroporini, and 1 species of a new genus of the dytiscid tribe Hyphydrini. The six species of *Phreatodytes* are classified into 3 species-groups, which show every step of reduction of the marginal furrows of the prothorax and elytra. Most archaic is a new species endemic to southeastern Kyushu, which looks like a miniature of *Metrius*. On the contrary, the type of the genus, *P. relictus*, and its new relative are most derivative as they have lost most parts of the furrows. This can be regarded as a positive proof that the Phreatodytidae is more primitive than the Noteridae and is independent from the latter. The three known species of *Morimotoa* form a rather compact group, which is evidently different from epigeal genera, while the hyphydrine new species is relatively similar in general conformation to epigeal species, with the obvious exception of the absence of eyes and the depigmentation. Other new species of phreatobiontic beetles should occur in the Japanese Islands, but after the 1950's, it has become extremely difficult to obtain new material because of rapid disappearance of driven wells.

11-055

MORTALITY FACTORS AFFECTING THE ARID DIAPAUSING DYTISCID *AGABUS DISINTEGRATUS* IN CALIFORNIA

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The dytiscid *Agabus disintegratus* breeds from fall to spring in seasonal wetlands in California. In the spring, teneral adults move to the water from embankment pupation sites. They feed and develop fat reserves prior to entering aestival diapause. As the habitat dries in the late spring, the adults take refuge at the soil-root interface of wetland plants where they pass the late spring, summer, and early fall in the absence of water. Adults cannot be induced to fly during diapause, but surface movement by some aestivating beetles is common during this dry period. Movement is apparently related to the quest for more optimal conditions. Mating and oviposition commence in the fall after inundation. Larvae develop through the late fall and winter and begin pupation in late February. Observed mortalities include an estimated 20% pupal parasitization by the ichneumonid, *Medophron* sp., and desiccation of adults prior to emergence and after aestival diapause. Mortality of larval populations will also be discussed.

11-056

THE *HYPHYDRUS* SPECIES OF THE ORIENTAL AND AUSTRALIAN ZOOGEOGRAPHICAL REGIONS (COLEOPTERA: DYTISCIDAE)

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The *Hyphydrus* fauna recorded from the Oriental and Australian zoogeographical regions is reviewed. At present 29 species belonging to five species groups are recognized. The distribution of the species is given on country-level and the origin of the different species groups is briefly discussed. A new *Hyphydrus* species from the Aru Islands in Indonesia is presented. The new species belongs to the species group *H. signatus*.

11-057

A NEW SPECIES OF *LACCOPHILUS* LEACH FROM EAST KALIMANTAN (COLEOPTERA: DYTISCIDAE)

Paolo Mazzoldi

A new *Laccophilus* belonging to the group *javanicus* Régimbart, recently collected in East Kalimantan (Borneo, Indonesia), is described. Habitus and genitalia of the new species are figured and diagnostic characters which differentiate it from the other species of the same group are illustrated.

The new species lives in small springs and in the first stretch of streams originating from them in primary rainforest.

11-058

AN ANALYSIS OF COMMUNITY-STRUCTURE AND HABITAT-PREFERENCE OF ADEPHAGE WATERBEETLES (COLEOPTERA: HALIPLIDAE, NOTERIDAE, DYTISCIDAE) IN CENTRAL EUROPE, USING 17 PHYSICOCHEMICAL PARAMETERS

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In a study of 60 random-sampled stagnant waters in Franconia the Hydradeephaga fauna was recorded semiquantitatively and 17 physicochemical parameters were analysed.

Using Canonical Correspondence Analysis TER BRAAK 1986, species data show distinct species assemblages, referring to 3 major types of waters:

- 1: bogs and acid, oligo- to mesotrophic woodpools: *bog-community*, with a sphagnophilic and an umbrophilic aspect;
- 2: polytrophic ponds and pools in open landscape: *pond-community*;
- 3: raw-bottom waters (sand-, claypits etc.): *raw-bottom-community*.

This three types of communities are „connected“ by transitions depending on succession in the waters. On the basis of 12 waters each recorded in spring, summer and autumn, it can be shown that the ordination of waters of the first two types is quite independent of season and therefore reproducible. In contrast raw-bottom pools show great seasonal variety in accordance with quick ecological succession.

In a Monte Carlo permutation test the 17 physicochemical parameters were tested for each parameters contribution to explanation of variation in species data. The test exhibits high significant ($p < 1\%$) or significant ($p < 5\%$) correlation for the parameters pH, conductivity, chloride, oxygene, carbonate hardness, total hardness, nitrite, sulphate, nitrate and temperature (in order of descending correlation).

The results are discussed towards building up an indicator-system for stagnant waters based on adephaga waterbeetles, especially for use in landscape ecology and monitoring.

11-059

WATER BEETLES (COLEOPTERA: ADEPHAGA) FROM EVACUATION ZONE OF CHERNOBYL NUCLEAR POWER STATION

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Present communication is based on the samples of water Adephaga which were collected during 1991-1995 near the Chernobyl Nuclear Power Station.

78 species of water beetles were found. They belongs to four families: Haliplidae - 7; Noteridae - 2; Dytiscidae - 62 and Gyrinidae - 7 species. Registration of 5 species, which at present period are still not recorded from other regions of Belarus (*Haliplus heydeni* Wehncke, *H. furcatus* Seidlitz, *Coelambus parallelogrammus* (Ahrens), *Agabus bifarius* (Kirby), *Gyrinus suffriani* Scriba) is of particular interest.

According to the results of our investigations one can state that radioactive contamination did not influence significantly beetle species diversity.

11-060

INVERTEBRATES OF THE TERRESTRIAL INTERSTICES IN AN ALPINE FLOODPLAIN
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Sediment baskets and tube-traps were designed to study the terrestrial arthropod community in gravel bar interstices along the Isar river (Bavaria, Germany). Sediment baskets filled with selected substrates were exposed at different depths (25 cm, 50 cm, 75 cm) in the gravel bed to allow for colonization. Tube-traps were installed to continuously collect animals moving through the sediments at 25 and 50 cm below the surface.

Initial results suggest that a specific arthropod community consisting of mites, spiders, springtails, the true bug *Cryptostemma alienum* Herrich-Schäffer (Dipsocoridae), larval Diptera, larval and adult Staphylinidae and Carabidae colonizes gravel bar interstices.

Different species of mainly surface active *Bembidion* use crevices in the gravel bed for hibernation. Hibernating beetles were collected down to a depth of about 1 m, preferably in coarse substrates just above the level of the subsurface flow.

11-062

ANIMAL COMMUNITIES ASSOCIATED WITH BAMBOO PHYTOTELMATA IN PENINSULAR MALAYSIA

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In recent years micro-ecosystems, for example, small water bodies held in terrestrial plants (phytotelmata), have increasingly been used to investigate fundamental biological problems. We found that bamboo phytotelmata are particularly suitable for studying the structure and dynamics of tropical animal communities. This ongoing investigation was begun in 1991 at the Ulu Gombak Field Studies Centre, near Kuala Lumpur.

Bamboo phytotelmata are created when the bamboo culm is punctured by insects or otherwise injured and then becomes filled with runoff rainwater. We first took a survey of the aquatic and terrestrial animal taxa inhabiting the internode cavity. Later on, we studied the spatial and temporal patterns of the animal community, the food webs and the life histories of some internode inhabitants. For the observation of the animals we used an endoscope.

We found that the internodes harbour a specialized fauna which mainly consists of arthropods. In the aquatic zone Diptera, especially mosquitoes, form the largest group of inhabitants. In the terrestrial zone a hitherto completely unknown animal community was discovered. Two remarkable features of the internode fauna are that some terrestrial predators, e. g. rove beetles or jumping spiders, are preying upon aquatic Diptera larvae and that representatives of many usually terrestrial taxa have adopted an aquatic mode of life. Furthermore, we found that different types of internodes, e.g., shoot internodes or decaying internodes, harbour different animal communities. In all, several hundred species were found, which makes the bamboo internodes among the most species-rich of all phytotelma-communities.

11-061

ARCTIC INSECTS:
DESICCATION VERSUS CRYOPROTECTION

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Many adaptations allow insects to survive the extreme conditions of arctic Canada. Some of these adaptations such as overwintering microhabitat selection, blood solute biosynthesis and body water content have been correlated in many ways with cold-hardiness. Such adaptations, however, may also contribute significantly to enhanced desiccation resistance and/or tolerance. The concerns that many of the ecological and physiological adaptations that have hitherto been interpreted as contributing to cold tolerance may also ensure winter survival to insects in cold, dry environments will be discussed.

11-063

FIRE AND WATER: COMPARISON OF THE ENVIRONMENT AND EVOLUTION OF CAVE-ADAPTED INSECTS IN VOLCANIC AND KARSTIC CAVES

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The discovery of cave-adapted insects in volcanic caves provides opportunities to test the evolutionary theories resulting from historic biospeleological studies in limestone caves. Compared to lava caves, limestone caves are usually older, deeper, larger, and more complex three-dimensional mazes, which connect with fewer mesocaverns, and which are often subjected to more complex geologic histories. Despite these major geologic differences, both volcanic and karstic caves harbor specialized insects that display similar adaptations, indicating that their evolution must be similar. Beyond the influence of entrances, the environment in subterranean habitats is similar in both cave types, being perpetually dark, humid, and nearly isothermal; lacking most environmental cues; and often containing lethal or sublethal gas mixtures and wet barren rocky substrates. Food resources differ greatly between lava and limestone caves, but the difficulty in finding food, as well as mates, are similar. The surface over both cave types is often at least partly barren, with food resources sinking into subterranean voids out of reach of surface species. Specialized cave animals evolved to exploit these resources in the harsh environment. Lava and limestone cave communities may accumulate and lose species differently over time. In limestone caves, succession progresses downward, with younger, energy-rich habitats deeper below the surface and relict unsuitable passages nearer the surface. Succession in lava caves is opposite, with younger habitats nearer the surface and older habitats deeper. In volcanically active areas, new lava flows continually rejuvenate aging cave habitats, allowing specialized species to occur in exceptionally young caves.

11-064

PHYLOGENY AND THE STUDY OF TROGLOBITIC TAXA

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As in every field of Comparative Biology (1), phylogeny provides an independent reference system in evolutionary studies on cave life to test current theoretical proposals (2, 3). Using phylogeny, sound hypotheses on the ancestral states of characters and their subsequent changes can be made by polarising the characters between related taxa (4, 5). Also hypotheses on evolutionary processes can be tested by comparing the patterns they imply with independently inferred phylogenetic patterns (6).

Two classical assumptions on the evolution toward troglobitic life have been analysed with this methodology : 1/ troglobitic taxa could be defined by troglomorphic characters, and 2/ troglobitic life would be an evolutionary dead-end.

Using phylogenetic analysis, so-called troglomorphic features may or may not occur in troglobitic taxa. These taxa can be characterised only by their behavioural ecology : they live, forage and reproduce exclusively in the subterranean environment. Preadaptations (exaptations) can also be precisely defined.

The evolution toward cave life does not appear to be a dead end. Two patterns subsequent to cave life appearance have been documented : speciation of troglobitic taxa in the subterranean environment, and reversal to an epigeal habitat. Troglobitic life thus appears as one step in clades diversification.

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11-066

SURFACE AND CAVE SPECIES OF *NOCTICOLA* (NOCTICOLIDAE): EVIDENCE FOR EVOLUTION OF NON-RELICTUAL TROPICAL TROGLOBITES

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Cave-adapted species of *Nocticola* were first discovered in caves of the Philippines in 1890, followed by additional cave and surface species of the family Nocticolidae from Africa, South Asia and Southeast Asia. According to the glacial relict theory of cave species evolution, obligate cave species should not have evolved in the tropics, due to lack of a major isolating mechanism that would have eliminated the troglomorphic parent species. Since the 1970's, many additional cave species of *Nocticola* have been collected from caves of Thailand, Malaysia, Indonesia and Australia. These include several highly cave-adapted species associated with "bad-air" caves. Epigeal relatives of the cave *Nocticola* have been found in Australia using light traps and intensive searches of forest floor. Presence of highly cave-adapted species of *Nocticola* in areas where surface relatives still exist can be explained by bioclimatic and adaptive shift models of cave species evolution.

11-065

THE HAWAIIAN CAVE PLANTHOPPERS (HOMOPTERA CIXIIDAE) - A MODEL FOR RAPID SUBTERRANEAN SPECIATION?

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After the successful colonization of a single ancestral species in the Hawaiian Islands, the cixiid genus *Oliarus* underwent intensive adaptive radiation resulting in 80 described endemic species. *Oliarus* habitats range from montane rain forests to dry coastal biotopes, and to subterranean environments: at least 7 independent evolutionary lines have adapted to lava tubes on Molokai (1), Maui (3), and Hawaii Island (3). Behavioral and morphological studies on one of these evolutionary lines on Hawaii Island, the blind, flight- and pigmentless *Oliarus polyphemus* have provided evidence for the reproductive isolation between allopatric populations which may in fact be separate species. Significant differences in song parameters were observed even between populations from neighbouring lava tubes, although the planthoppers are capable of underground migration through the voids and cracks of the mesocavernous rock system which is extant in young basalt: after a little more than 20 years, lava tubes within the Mauna Ulu 1974 flow had been colonized by *O. "polyphemus"* individuals, most probably originating from a near-by kipuka. Amazingly, this species complex is found on the youngest of the Hawaiian Islands, with probably less than 0.5 m.y., which suggests rapid speciation processes. Field observations have led to the development of an hypothesis to match underground speciation with the dynamics of vegetational succession on the surface of active volcanoes: planthopper range partitioning and geographic separation of populations by young lava flows, founder events and small population size may be important factors involved in rapid divergence. Approaches to test this hypothesis are introduced and discussed.

11-067

CLIMATIC FLUCTUATIONS AND TROPICAL TROGLOBITIC EVOLUTION

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Allopatric speciation of cave organisms requires geographic isolation as a result for instance of local extinction of epigeal populations. A classical model proposed to explain the origin of terrestrial troglobites in temperate zones relates this local extinction to long-term climatic fluctuations, such as Pleistocene glacial cycles. From this model, it is predicted that: 1) Regions subject to more frequent and accentuated fluctuations will present a higher ratio troglobitic/troglophilic species, especially in the case of terrestrial fauna. 2) Dry, arid or semi-arid areas will bear the most specialized troglobites.

This model can be applied to the origin of tropical troglobites as well, especially for terrestrial organisms. Evidences of Quaternary climatic changes in tropical South America are indisputable, indicating drier climates related to glacial periods. Moreover, preliminary comparative data on the composition of Brazilian cave fauna seems to fit those predictions.

For instance, relatively few terrestrial troglobites are found in those areas in Central Brazil which remained covered by a cerrado vegetation similar to that of the present time during the last glacial period.

Highly specialized troglobites and some "relictual" hygrophilic organisms have been found in caves of northeastern Brazil (and in the Argentinian Patagonia). These animals may have colonized the subterranean biotope during the short periods of higher humidity (possibly near the interglacial maxima) and became isolated for long periods since then (including the glacial and part of the interglacials).

Less specialized troglobites are found in areas now covered by humid vegetation but that had dried during glacial periods, as seems to be the case of the southeastern Brazil. It probably happened because the total time of isolation is shorter (all or part of the glacial).

11-068

CARABID BEETLES AND SUBTERRANEAN ENVIRONMENTS

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Carabid beetles, polyphagous predators and/or omnivores, are a successful, highly diverse group of Coleoptera, represented in a wide range of biomes, from temperate to tropical and boreal regions. Mostly winged, often arboreal in tropical forest communities, they became true "ground beetles" in temperate lands: in these countries, many species are brachypterous, montane, nocturnal, often steno-hygrophilous soil dwellers. These are excellent conditions for a pre-adaptation to the subterranean environment.

With the exclusion of the heliophylous (Cicindelinae) or phytophagous (Harpalinae, and Amarini plus Zabritini among Pterostichinae) elements, several groups of carabid beetles colonise different subterranean compartments (endogean, upper hypogean zone and caves). Large numbers of subterranean species are included in the Clivinini Reicheiina, Anillini, Trechini, Platynini, Sphodrini, Pterostichini and Molopini. A few highly specialised (upper hypogean and/or cave dwelling) species are found also in the tribes Ozaenini, Calleidini, Zuphiini and Brachinini, but the largest number of such specialised elements belong to the tribe Trechini.

Distribution patterns of subterranean carabids are not homogeneous throughout the world. The areas with the highest diversity in specialised elements are the boreal and austral temperate 'mediterranean' zones while tropical lands are particularly rich in endogean species.

11-070

INTRODUCTION TO PHYLOGENY AND EVOLUTION OF SUBTERRANEAN AND ENDOGEAN CHOLEVIDAE (= LEIODIDAE CHOLEVINAE)

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This is an introduction to phylogeny and evolution in subterranean and endogean Cholevinae, a group within the Staphylinoid assemblage of beetle families. I review ideas on the monophyly of the family groups and prefer to use an expanded family Leiodidae; containing the subfamilies Camiarinae, Catopocerinae, Leiodinae, Coloninae, Platypsyllinae, and Cholevinae. These groups have very diverse biologies. Most are scavengers or fungal feeders, but some are ectoparasites. Some show clear and ancient "transantarctic" distributions. The Cholevinae represent the world's greatest (after Carabidae) endopterygote insect radiation in soil and cave habitats. I review some of the adaptations, morphologies, and biologies of this interesting fauna, drawing mostly from European and North American examples. Topics of productive future research are suggested.

11-069

HISTORICAL BIOGEOGRAPHY OF JAMAICA: EVIDENCE FROM CAVE INVERTEBRATES

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The biogeographic history of the West Indies, and Jamaica in particular, is controversial. This paper examines historical clues in the invertebrate cave fauna of Jamaica. Cave faunas are frequently considered to be archaic and relictual, and thus could provide possible clues to early regional biogeographic history. The troglobitic (cave specialized) fauna contains (1) 12 freshwater-brackish water species (mostly crustaceans) that have invaded from the sea, and (2) 25 species of terrestrial arachnids and insects. Cladistic analyses suggest a closer relationship to Central America than to Cuba-Hispaniola. There is no strong evidence for a vicariance origin of the fauna in a continuously subaerial environment since separating from proto-middle America. Over-water dispersal seems to account for the presence of the ancestors of the troglobitic fauna in Jamaica, and they probably arrived after Jamaica became emergent, about 20 million years ago.

11-071

CONSIDERATIONS ON THE PRESENT KNOWLEDGES ABOUT THE ITALIAN CHOLEVIDAE AND THEIR DISTRIBUTION, WITH PARTICULAR REFERENCE TO THE HYPOGEAN SPECIES (COLEOPTERA)

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The Italian Cholevidae fauna includes at present some 265 species (187 of which only belong in Leptodirinae) arranged in 50 genera. An overview of each genus is given showing some of the main aspects of the biology and the distribution both of each genus in its whole and of some more significant species.

Referring to the major groups, it is reported that: - Nemadinae have in Italy only one central-european species; - Ptomaphaginae include some central-european species as well as some W-mediterranean, circumadriatic or endemic ones; - Anemadinae are, in the whole, mediterranean with two endemic species; - Cholevini include principally central-european or european species; also some endemic species are present; - Catopini are represented by several european or central-european species, but also olarctic, palaearctic, mediterranean, S-mediterranean or endemic taxa occur; - Leptodirinae have, in general, endemic genera, being Bathysciola, Parabathyscia and Speonomus only with a wider distribution.

All the Italian Cholevidae are related to the soil fauna; Leptodirinae include a lot of endogean or hypogean species, while only some representatives of other subfamilies occur more or less regularly in cave environment.

Some aspects of the systematic and distribution of the Italian Cholevidae are discussed.

11-072

CLADISTIC BIOGEOGRAPHY OF CAVERNICOLOUS
PTOMAPHAGUS (LEIODIDAE: CHOLEVINAE:
PTOMAPHAGINI) IN THE UNITED STATES

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Cavernicolous species of *Ptomaphagus* occur on three continents; most occur in the southeastern United States. This paper examines the 18 species of the *P. hirtus* species group of troglobites in the southeastern USA. Predictions of the speciation models, cladograms, and area cladograms suggest (1) periods of successive glacial overland dispersal, and (2) cladogenesis by climate induced (vicariant) interglacial isolation in caves. Adjacent and non-overlapping (parapatric) ranges of the species show the possibility of competitive exclusion for the prevention of sympatric distributions.

Vicariance Biogeography is a method of construction of general area cladograms to attempt to learn the histories of regional biotas. *Ptomaphagus* is proposed as a model for use in seeking general-area cladograms of geographic patterns in southeastern US terrestrial cave faunas. Have the terrestrial cave faunas been assembled by climatically coordinated community vicariance events (as predicted when general area cladograms can be found)? Or, have cave faunal assemblages come together in a random (non-coordinate) way, controlled by multiple external physio-geographic barriers? Is the biogeographic history of cave faunas in general that of (1) individualistic random (non-predictive) dispersal events, or (2) coordinated vicariance biogeography? Cladistic analyses of many components of regional cave faunas are necessary to answer such questions. Because of the similar ecological requirements of terrestrial cave faunas, they should be a good model system for testing principles of vicariance biogeography.

11-074

A SYSTEMATIC REVISION OF *ADELOPSIS* AND COMMENTS ON
THE PHYLOGENY OF THE PTOMAPHAGINI
(COLEOPTERA, CHOLEVIDAE)

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The genus *Adelopsis* was previously defined to include the Neotropical species of the Ptomaphaginae (presently 25 species) with aedeagus different from that of *Ptomaphagus*. Afterwards, it was redefined with the description of 16 species from the Appalachians (USA) in 1978. The recent study of Brazilian cavernicolous species warned about the paraphyletic condition of the genus and the need of a systematic revision. The study of the large collection of S.B. Peck, from Canada, led to the recognition of more than a hundred undescribed species and corroborated the paraphyly of the genus.

A cladistic analysis was made based on characters studied from several species in the genera of the tribe and allied cholevids. The resulting cladogram helped in the definition of four new genera and the redefinition of the previous recognized genera. Some previously described species of *Adelopsis* are transferred to the new genera. The group of Appalachian species of *Adelopsis*, as well as the genera *Echinocoleus* and *Synaulus*, are defined as subgenera of *Ptomaphagus*. The subtribe Ptomaphagina, which includes *Ptomaphagus* and *Proptomaphagus*, resulted to be paraphyletic.

In these new definition, the genus *Ptomaphagus* includes several probably independent lineages with subterranean evolution, including endogean, myrmecophilic and troglitic species, some showing highly evolved related morphology. In the other genera, as in *Adelopsis*, *Proptomaphagus*, and *Ptomaphagus*, some species also colonized the subterranean environment, although only few show eye, wing and/or pigmentation reductions. In *Adelopsis*, these reductions seem to be specially related to litter inhabiting species.

11-073

CAVE AND ROCK DEBRIS DWELLING SPECIES OF THE
CHOLEVA AGILIS SPECIES GROUP FROM CENTRAL
EUROPE (COLEOPTERA: LEIODIDAE: CHOLEVINAE)

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In central Europe, five (from the total number of 11) species of the *Choleva agilis* species group have been known: widely distributed epigean *C. agilis* (Illiger, 1798); *C. lederiana* Reitter, 1901 (S Finland and NW Russia; epigean as well as cave records); *C. septentrionis* Jeannel, 1923 (S Norway and Sweden, and also Krkonoše Mts., N Bohemia; mostly epigean records, alpine stone fields in Krkonoše Mts.); and two strictly cave dwelling species: *C. holsatica* Benick & Ihssen in Benick, 1937 (Segeberger Höhle cave, N Germany) and *C. gracilentia* Szymczakowski, 1957 (caves in Sokole Góry Mts., SW Poland).

Recently, three other isolated populations with relict distribution have been found (presently undescribed), in: (1) cold taluses of České středohoří Mts., N Bohemia; (2) caves in Pilis Mts., NW Hungary; (3) cave in Bakony Mts., W Hungary.

Multivariate statistical analyses of 15 morphometric characters have been made for samples of all 8 species. The gradient in increasing length of appendages (length of antenna, metatibia and metatarsus) have been found from epigean to rock debris dwelling to cave dwelling species, probably as adaptations to hypogean conditions.

11-075

MAIN QUESTIONS IN PHYLOGENY AND BIOGEOGRAPHY
OF CHOLEVIDAE (COLEOPTERA)

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A phylogenetical system of Cholevidae (treated as a family) is presented, and relationships of them with other higher taxa of Staphylinoidea are discussed. Characters regarded as phylogenetically meaningful are reinterpreted, and their evolution in the family and in related groups are widely illustrated. Modifications of several features, due to adaptation to different environments and ways of feeding, are also made evident. A preliminary cladistic analysis of subordinate taxa of the family, in which the character state polarity is based on out-group comparison, is attempted. Furthermore, a hypothesis of the origin of different subfamilies, and a zoogeographical history of the Cholevidae as a whole, are proposed.

11-076

PHYLOGENY AND BIOGEOGRAPHY OF CHOLEVIDAE LEPTODIRINAE (COLEOPTERA)

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Among the subfamilies of Cholevidae, Leptodirinae present the most specialized and impressive adaptations to the subterranean environment. For that reason, several convergent adaptive features are present in different genera and/or phyletic lines not directly related each other. Apart from some well known characteristics common to other hypogean beetles, such as reduction or loss of eyes, depigmentation, loss of metathoracic wings, elongation of antennae and legs, other peculiar modifications of the body such as pseudophysogastry, elongated and prognathous head, modified mouth parts in relation to the way of feeding in oligotrophic caves, are noticeable. So, it is evident that any phenetical approach is impossible to achieve a phylogeny of the group.

In the present research, characters regarded as phyletical meaningful, their weight and state polarity based on outgroup comparison, are discussed and reinterpreted, for a cladistic analysis and a congruent zoogeography of the subfamily in the Euro-Mediterranean area.

11-078

ZOOGEOGRAPHICAL REVIEW OF THE TROGLOBITIC AND ENDOGEAN CARABIDAE AND CHOLEVIDAE (COLEOPTERA) OF YUGOSLAVIA AND ADJACENT REGIONS

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An updated zoogeographical review of the most diverse subfamilies of Balkan cavernicole and endogean coleopterans -- Bembidiinae, Trechinae (Carabidae) and Leptodirinae (Cholevidae) is based on the revised literature data, as well as on the abundant new material collected during the last 20 years (from Serbia and Montenegro, mainly unpublished). A synoptic review of the numbers of genera, species and subspecies is presented, as recorded in different countries of the region. The distribution of species was analysed according to their life form and ecological preferences (troglobitic, troglophile and endogean, respectively). For the territory of the former Yugoslavia, distributional patterns of the recorded genera, as well as the types of the species range endemicity are discussed. Within the territory of F.R. Yugoslavia (Serbia and Montenegro), a comprehensive faunistic review is based on 84 described and about 30 new species, with emphasis on the notified local differences in species diversity. The status and significance of the apparent diversity centres were considered in relation to the regional trends of the expected taxonomic richness, with respect to their relevance for the biodiversity conservation.

11-077

PHYLOGENETIC PROBLEMS, CURRENT CLASSIFICATION AND GENERAL CATALOG OF WORLD LEIODIDAE (INCLUDING CHOLEVIDAE)

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Subterranean and endogean cholevines are placed in the context of a world-wide review of the past and present classification of related Staphylinoidea. Arguments are presented for recognizing a single large monophyletic family, Leiodidae, rather than a series of less well-defined families including Cholevidae, Leptinidae, etc. Six leioidid subfamilies are recognized, all but the first probably being monophyletic: Camiarinae, Catopocerinae, Leiodinae (=Anisotominae), Coloninae, Cholevinae (=Catopinae, Leptodirinae) and Platypsyllinae (=Leptininae). Outstanding phylogenetic problems at the subfamily and tribal levels are discussed.

The Cholevinae contain the greatest diversity in subterranean habitats, with some 140 genera and 600 species in the Leptodirini (=Bathysciini) in the Palaearctic Region.

Keys for identification, diagnoses, and brief summaries of the biology of each subfamily and tribe are given. A complete generic catalog, with indication of genera examined and the distribution and number of named species for each genus, and an appendix of new taxonomic and nomenclatural changes, are also provided.

11-079

TROGLOBITIC ADAPTIONS AND PHYLOGENETIC AGE OF A POST PLEISTOCENE CAVE BEETLE IN NORTHERN GERMANY (CHOLEVA HOLSATICA IHSENSEN & BENICK; CATOPIDAE)

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Troglobitic adaptations and ecological basis of Choleva holsatica IHSENSEN & BENICK (Catopidae, Coleoptera) were studied (for example eye-analyses by REM-method and factor-gradient-tests in the laboratory). This species is endemic to a climatically constant cave (9°C, 100 % humidity) in Northern Germany. Its closest surface relative is Choleva septentrionis Latr., which occurs in Norway and from which it was probably separated subsequent to postpleistocene climatic warming.

11-080

IMPACT OF TEMPERATURE CONDITIONS ON THE REPRODUCTION OF AQUATIC BUGS (HETEROPTERA: CORIXIDAE)

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Field and experimental works were carried out on 5 species of Corixidae (*Sigara lateralis* (Leach), *S. falleni* (Fieber), *S. striata* (Linnaeus), *S. cocinna* (Fieber), *S. praeusta* (Fieber)).

First eggs were laid when the temperature was between 10° and 12°C. It seems that these temperature interval represents lower ones for egg-laying in Corixidae. During the temperature increase from 12° to 25°C rate of egg-laying grew steadily reaching the maximal values at 25°C.

The study of the impact of temperature on the total fecundity was carried out on 5 species of Corixidae. Total fecundity is relatively conservative reproduction index. It is not affected by the temperature factor and apparently is determined genetically. Of course, optimal temperature conditions (15-25°C) for egg-laying in investigated species of Corixidae are necessary for its realisation.

11-081

EVOLUTION OF THE THORACICO-ABDOMINAL JUNCTION IN THE NOTONECTOIDEA AND PLEOIDEA (HETEROPTERA, NEPOMORPHA)

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The lateral thoracico-abdominal regions of twenty two species of eleven genera of the superfamilies Notonectoidea and Pleoidea (Notonectidae: *Notonecta*, *Enithraes*, *Aphelonecta*, *Nychia*, *Martarega*, *Anisops*, *Buenoa*; Pleidae: *Plea*; Helotrephidae: *Helotrephes*, *Distotrephes*, *Trephotomas*) were studied.

The morphological features of the pterothorax and base of abdomen are partly influenced by the locomotion, feeding and respiratory strategies, and by the pteropolymorphism too. Nevertheless, these have significant differences on the taxonomic level of tribes, subfamilies and families. Some evolutionary trends of the arrangement of the lateral pterothoracic region and thoracico-abdominal junction were ascertained in the particular taxons. A hypothesis on the origin and homologization of the individual parts of thoracico-abdominal junction was expressed.

11-082

EFFECTS OF GRADUAL INCREASE IN PHOTOPHASE ON DISPERSAL POLYMORPHISM AND REPRODUCTION IN A WATER STRIDER, *AQUARIUS PALUDUM* (F.) (HETEROPTERA: GERRIDAE).T. Inoue and T. Harada¹

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Constant long-day conditions induce brachypterous form and reproduction, whereas constant short-day conditions cause macropterous form and diapause in *Aquarius paludum* (F.).

The critical daylength for the determination of wing forms is about 13.75 h, and that for reproduction is 13 h in Kochi (33° 33' N, 133° 35' E) (Harada & Numata, 1993). The photophase was increased by 2 or 3 min every 2 days (12.25 h to 13.25 h, or 11.5 h to 12.5 h) during the first to the fourth (penultimate) nymphal instars. The increase had no effects on both wing form and maturation of oocytes. However, it inhibited flight behavior and caused higher proportion of adults which histolyzed their mesothoracic flight muscles before the 30th imaginal day than did a constant daylength of 13.25 h or 12.5 h.

The results suggested a following hypothesis: Increase in photophase is converted to another endocrine pathway excluding juvenile hormone which may induce brachypterous form and reproduction, and the pathway causes the inhibition of flight behavior and histolysis of flight muscles in *A. paludum*.

11-083

Ecological and morphological differentiation of the two closely related species *Gerris asper* and *G. lateralis* (Hemiptera, Gerridae).

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Although the two closely related species *Gerris* (*Gerriselloides*) *asper* and *G. lateralis* differ in their distribution areas and habitats, they were often confounded among each other and with other *Gerris* species. Up to now the five larval stages of the two species could not be discriminated.

Whereas *G. lateralis* has been reported from the Swiss Jura Mountains some twenty years ago, *G. asper* was only recently recorded on the Swiss Plateau. Investigations were made on the ecological differentiation of *G. asper* and *G. lateralis*. The five larval stages were differentiated morphologically. Our regular samples suggest that the populations of *G. asper* on the Swiss Plateau are generally univoltine. Field experiments on inter- and intraspecific competition in the Swiss Jura Mountains and on the Swiss Plateau show, that under interspecific competition *G. lateralis* is superior to *G. asper* at both locations.

The five larval stages of *G. asper* and *G. lateralis* are described and differentiated by various pigment patterns and also by morphometric features. Larvae of *G. asper* are more delicate than those of *G. lateralis*. In contrast with larvae of *G. asper* the larvae of *G. lateralis* show dark epimeral and episternal lobes and also dark adjacent ventral and lateral areas. Using linear discriminant analysis and stepwise backward elimination the number of morphometric measurements was reduced from eleven to the two measures head width and length of antennal segment 4. Applying a single discriminant function common to all 5 larval stages, 95 % of the larvae can be determined.

11-084

THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE DISTRIBUTION OF HEMIPTERA OF ACID HEATHLAND FENS
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Heathlands have become an increasingly scarce habitat. Because they grow on acid and nutrient-poor sandy soils, they impose particular demands to the inhabiting fauna and flora. Until now, only few biocoenological investigations have been undertaken on the species living in acid heathland fens. Here, we investigate the distribution of Hemiptera in relation to environmental parameters. Hemiptera were collected in 31 pools at the "Groot Schietveld" near Brecht (northern Belgium) during the summer of 1993. Several biotic and abiotic habitat characteristics were measured.

In total 26 species were found belonging to seven families: Corixidae (16sp), Gerridae (2sp), Hydrometridae (1sp), Naucoridae (1sp), Nepidae (2sp), Notonectidae (3sp), Veliidae (1sp). Five species, *Corixa dentipes*, *Cymatia bonndorffi*, *Micronecta meridionalis*, *Sigara limitata* and *Microvelia reticulata* are rare and one, *Glaenocoris propinqua*, is very rare in Belgium.

Multivariate techniques (canonical correspondence analysis, CCA, and two-way indicator species analysis, TWINSpan) were applied to examine the impact of environmental variables on the occurrence of species. CCA showed that pH, plant cover, hardness, O₂ and depth explained 92.5% of the total variance of the species distribution. The Hemiptera species richness (number of species) and the diversity (Shannon-Wiener index) decreased with increasing amount of vegetation cover, depth and acidity.

11-086

THE DISTRIBUTION AND ABUNDANCE OF PTERONARCYS CALIFORNICA NEWPORT IN THE MADISON RIVER, MONTANA, USA

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Despite the importance of *P. californica* to river community ecology, little is known about its distribution and abundance in the Madison River, MT, USA. Larval *P. californica* in the section of the Madison River downstream of the top release dam grow faster and larger but are much less numerous than in the section of the Madison River above the dam. Habitat availability seems to effect their abundance. Other dam-related factors may also contribute to *P. californica* distribution and abundance. Methods used in this study for counting shed exuviae may be used by others to monitor water quality locally.

11-085

SPATIAL DYNAMIC OF THE CADDISFLY (TRICHOPTERA) COMMUNITIES OF A BASIN IN THE NORTH-WEST OF SPAIN

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The spatial dynamic of the caddisfly (Trichoptera) communities in the Sil Basin (Spain) was analysed and compared with the structural change of the riparian and channel vegetation. The study was based on the variation of specific diversity (Shannon index) at 40 localities, which were sampled in summer. The structural heterogeneity analysis and the evaluation of the specific composition changes between consecutive sampling sites were also carried out.

The Sil River Basin is an hydrographic network situated in the north-west of the Iberian Peninsula. The basin is characterized by its environmental heterogeneity caused by the strong contrast between altered and unaltered rivers.

The average diversity of caddisfly communities for the different rivers was relatively low, not above 2 bits/individual. We have not found correlation between heterogeneity and geographic distance in the macrophyte and caddisfly communities. The influence of coal mining and urban pollution is shown by important spatial changes in the Trichoptera community structure, and therefore high values of heterogeneity. However, the heterogeneity of the macrophyte communities is basically related with the morphologic variability of the channel and the banks.

11-087

MORPHOLOGY AND COLEOBIOSIS OF TWO SPECIES OF HELICOPSYCHE von Siebold, 1856 PRESENT IN ITALY (INSECTA TRICHOPTERA: HELICOPSYCHIDAE).

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The genus Helicopsyche has a worldwide distribution, most of the species being found in intertropical areas. In Europe, 5 species have been described. In Italy up to now, 2 species have been found: H. sperata in the pre-Alps (including Canton Ticino), the Apennines, Sicily, Elba Island and H. revelieri in Corsica, Sardinia, Capraia Island. The aquatic stages, characterized by snail-like cases of sand grains (diameter up to 5 mm) are found in springs, hygropetries and margins of shallow streams. During the early instars, the larva builds a curved tube-case which later becomes a helical case. At the last instar, the case is coiled three times, around a columella, lined with pillars of vertically-stacked sand grains bordered by silk threads. The sand grains are connected together by a loose irregular silk weave (under scanning electron microscope). An opening on the upper coil facilitates water circulation. The larva, 5-6 mm in length, is coiled inside the case in such a way that the posterior end lies above the head. The dorsal protuberance of the first abdominal segment is larger and more prominent in H. revelieri. Gills are absent and fork-like plates occur on the sides of segment 8. The anal claw has a sclerified comb-like structure. The silk glands are double-folded and symmetrically located in the abdomen. The pupal case is closed by a thick woven silk lamina with a thin transverse opening.

11-088

NEW DATA ON THE TRICHOPTERA OF A FEW SPRINGS OF THE PROVINCE OF BERGAMO - LOMBARDIA.

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In this report we present the data relative to the Trichoptera found in 15 springs of the Province of Bergamo (Lombardy) from 350 to 1,800 metres of altitude.

This study has been carried out in the period 1993-1995 and has led to the identification of 64 species from 4,000 adult insect specimens examined, that is particularly interesting in consideration of the very few data available related to this area of the Alps.

It is particularly interesting the presence of some species with a not very well characterised distribution such as *Microptila minutissima* Riss for which these are the only data available for Italy. Several specimens of the stenodemite of the Central Alps *Rhyacophila hirticonis orobica* Moretti were also found, confirming the characters of the holotype but requiring a more detailed comparative analysis to establish the exact taxonomic status. For ten other species our findings represent the first description for the Lombardy region. Our data confirm that this, sector of the Alps despite not being well investigated is particularly interesting as it represents the limit of longitudinal distribution of several taxa both from the East and from the West. For instance, we found *Tinodes dives dives* Pict., (a taxon of Central Western Europe) and of *T. d. jeekelii* Bots. (typically from the Eastern Alps and the Balkans), as well as some intermediate forms.

From our data it has been possible to assess the abundance of the different species in the various springs and to perform some quantitative analysis.

The method of Sørensen has allowed the correlation of the trichoptero-coenosis with the altitude of the various sites.

11-089

ZONATION OF HYDROPSYCHIDAE (TRICHOPTERA) IN THE SIMETO RIVER (SICILY)

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The Hydropsychidae species inhabiting the Simeto River, the biggest river in Sicily, and its principal mountain branch, the Saracena Stream, were studied.

The sampling of the aquatic stages was carried out about every two months between 1992 and 1994. In order to obtain comparable data, the sampling time for each site was about 20 minutes.

Seven species of Hydropsychidae were found: *Hydropsyche dinarica* Marinkovic-Gospodnetic, 1979; *H. doehleri* Tobias, 1972; *H. klefbecki* Tjeder, 1946; *H. modesta* Navas, 1925; *H. morettii* De Pietro, 1996; *H. spiritoi* Moretti, 1991; *Cheumatopsyche lepida* (Pictet, 1834). Only three species (*H. morettii*, *H. modesta* and *C. lepida*) were found in the low course of the Simeto River.

Regarding the ecological zonation of Illies and Botosaneanu (1963), the species showed the following preferences: *H. doehleri* for hypocrenal and epirhithral; *H. dinarica* for epirhithral; *H. klefbecki* and *H. spiritoi* for metarhithral and epirhithral; *H. morettii* and *C. lepida* for hyporhithral; *H. modesta* for epipotamal.

The preferences in the ecological zonation, which showed no direct correlation with altitude, were confirmed in the populations of other Sicilian streams.

11-090

PLANT, FUNGAL OR ANIMAL DIETS: DIFFERENT EFFECTS ON LARVAL DEVELOPMENT OF MOSQUITOES (DIPTERA: CULICIDAE)

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Mosquito larvae colonize small shallow freshwater pools with usually unbalanced nutrition, in quantity as well as in quality. Compared with food of microbial or animal origin, plant material is poor in protein, lipid and vitamin content or, such as seeds, largely inaccessible to mosquito larvae. Mosquitoes have evolved into various ecotypes which are adapted for efficient development despite of dietary imbalances.

In the field it is difficult to separate the various nutritional components and their individual significance as dietary requirements. Therefore, we have experimentally selected three diets of plant, fungal, and animal origin. We quantified the effects of nettle powder, brewers yeast, and TetraMin® on larvae from different mosquito species. In contrast to animal food, development was decelerated and population size, body size, and caloric reserves were reduced with plant and fungal food, respectively. In addition, nettle powder affected the metabolism of female mosquitoes as reflected by reduced hatching of their offspring. This effect could largely be compensated by the female through repeated blood meals.

On a higher trophic level, i.e. mosquitoes whose larvae prey upon other larvae, pupal mortality of the predator was higher and fecundity was significantly lower when their food has been raised with plant material. Our data support the role of essential fatty acids in development and reproduction, as reported in the literature.

Supported by the Swiss National Science Foundation.

11-091

NEW DATA AND FAUNISTIC ANALYSIS OF THE HYDRAENIDAE (COLEOPTERA) OF ANDALUCÍA (SOUTHERN SPAIN).(*)

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A taxonomic study was carried out on 1243 coleopteran specimens belonging to *Hydraenidae* from several localities of Andalucía (Southern Spain). A total number of 25 species were identified and eight are firstly reported for four provinces. From these results and the reported literature we were able to elaborate an updated *Hydraenidae* species list and to perform faunistic and corological studies for Andalucía.

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11-092

INFLUENCE OF ENVIRONMENTAL VARIABLES ON THE DISTRIBUTION OF ELMIDAE AND HYDRAENIDAE IN A RIVER BASIN IMPACTED BY MINING ACTIVITIES (COLEOPTERA)

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Fourteen species of Hydraenidae and thirteen of Elmidae were collected from riffles of several rivers in the Sil River basin (NW Spain). The aim of the research was to establish the potential influence of coal mining activities, the main impact in the study area, on the distribution of these species.

Several parameters were analysed by means of Canonical Correspondence Analysis (CCA). The results reveal that distance from the source and mining activities are the most significant factors.

CCA allowed to define groups of species with different degrees of tolerance to pollution derived from mining.

11-093

A CLASSIFICATION AND EVALUATION OF WATER BEETLE ASSEMBLAGES IN THE SIBILLINI NATIONAL PARK (CENTRAL ITALY).

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Water beetles were recorded from different sites in the "Pian Grande" inside the Sibillini National Park and assembled for analysis. The main water beetle assemblage were identified by multivariate analysis and then habitats of these assemblages were characterized by measurement of a range of physical and chemical factors. Each taxon is linked with the environmental variables and the ordination of the taxa and variables allows presentation of the more synthetic correspondence between the Coleoptera and their ecological and biological characteristics.

11-094

THE GENUS *HYDROCHUS* (COLEOPTERA:HYDROPHILOIDEA:HYDROCHIDAE) IN SOUTH AMERICA

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Twenty-three South-American species of the genus *Hydrochus* were examined, seeking for a natural division of the genus. Male genitalia suggests four species-complexes, with a few species which appear to have evolved along independent paths. Most species are exclusive of South America; two range from Central America to Argentina; these belong to the largest complex, which centers around *H. obscurus*. A second complex is made up of the small-sized species found in tropical and subtropical South America. The third comprises *H. secretus* and two similar species; the fourth *H. metallipes* plus *H. ducalis*. For the two last complexes, some external characters had to be taken into account. Use of new characters is becoming necessary for more natural grouping. Examination of the microtrichia on the inner elytral face under Scanning Electron Microscope is suggested as one possibility

11-095

NUMBER OF INSTARS IN *SIPHONURUS AESTIVALIS* (EPHEMEROPTERA, SIPHLONURIDAE): TEMPERATURE AND FOOD INFLUENCES

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To determine the precise number of instars in mayflies, a Palmen Body Standard Method was developed, based on the usual techniques applied in histology. This technique is successfully tested on mature larvae of *Siphonurus aestivalis* (Ephemeroptera, Siphonuridae) growing in two biotopes, a pond and a river with different temperature regimes and food availabilities.

The number of larval instars for *S. aestivalis* is included between 10-17 for the specimens coming from the pond and 12-17 for the individuals collected in the river. Considering each intralayer space of the Palmen body as the developmental time of each larval instar, the moulting frequency in the two biotopes is compared. The importance of water temperature and food on the moulting number is discussed.

11-096

DRIFT OF LOTIC MAYFLIES IN STREAMS WITH CONTRASTING PREDATION REGIMES: FIELD PATTERNS AND BEHAVIOURAL INTERACTIONS.

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Nocturnally biased drifting of stream invertebrates is often regarded as a behavioral mechanism whereby invertebrates avoid encounters with visually hunting fish predators. To examine this hypothesis, we collected invertebrate (especially mayfly) drift from five streams in northern Finland: one with trout (drift-feeding fish), one with sculpin (benthivore), one with both species, and two fishless streams. Drift of *Baetis* mayflies was aperiodic or slightly diurnal in both fishless streams on all sampling occasions. In streams containing fish, the peak in drift in July occurred during the short crepuscular period around midnight. At other sampling times, drift was clearly nocturnal in streams with trout and, to a lesser extent, also in the stream lacking drift-feeding fish. It thus appears that nocturnally constrained drifting may also arise as a post-contact mechanism: invertebrates enter the water column after being encountered (and possibly attacked) by a benthic-feeding fish. We tested this in laboratory trials where mayflies were exposed to sculpin predation. Sculpin were almost exclusively nocturnal, and their foraging activity resulted in a much higher frequency of drift entries by mayflies at night than during the day. We suggest that nocturnally restricted drifting of stream invertebrates may function either as a pre- (avoidance of visual predators) or a post-contact (flight after encounter) antipredatory mechanism.

11-097

THE DRIFT OF BENTHIC INVERTEBRATES OF THE VERA STREAM (ABRUZZO-CENTRAL ITALY)

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The results of pluriennial investigations about invertebrate drift in a small, cold-water stream in Gran Sasso (Abruzzo - central Italy) massif are reported. This study is carrying on benthic invertebrates and especially on four taxa (Plecoptera: *Protonemura*, Ephemeroptera: *Baetis*, Diptera: Chironomidae, Crustacea: Gammaridae), collecting drift and benthos samples. Effects of seasonal fluctuations and carrying-capacity variations on drift of benthic invertebrates are analyzed.

11-098

ON THE SCALE INSECTS FAUNA (HOMOPTERA, COCCINEA) OF THE WHITE SEA INTERTIDAL ZONE

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The scale insects fauna of the intertidal zone of the White Sea was investigated for the first time. 4 species were found. *Acanthococcus* sp. and *Trionymus* sp. inhabit above-ground parts of plants and settle on places flooded approximately once a week. *Atrococcus craccens* Will. and *Rhizoecus halophilus* (Hardy) live on roots and can develop successfully in the zone submerged twice almost every day. At the White Sea the rare itself phenomenon of the scale insects inhabitation on flooding sea shore combines with one of the northernmost occurrences of the Coccinea.

11-099

THE FIRST STUDIES ON THE ENTOMOLOGICAL FAUNA OF THE PERSISTENT SNOW-ENVIRONMENTS IN THE DOLOMITES MOUNTAINS: BRACHYPTEROUS INDIVIDUALS OF *DIAMESA STEINBOECKI* GOETGH. (DIPTERA: CHIRONOMIDAE).

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During a study carried out on the entomological fauna of the Dolomites Glaciers we have found the presence of *Diamesa steinboeckii* Goetgh., the glaciers-fly, a dipteran with short underdeveloped wings.

Among the population of these insects a great variability of development degree has been found in their wings both in the sizes and in their shapes. In this study we compare the thorax muscular structure with the different kinds of wing development.

We also made some considerations about the morphological relationships between the body different parts and their adaptation to the life in the extreme environments such as the high altitude glaciers.

11-100

AQUATIC INSECTS OF THE KÖYCEĞİZ-DALYAN NATURE RESERVE AREA WITH THEIR USAGE,EVALUATION OF ENVIRONMENTAL QUALITY OF RUNNING WATERS

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The Köyceğiz-Dalyan Region is an important aquatic ecosystem in South Western Turkey. From the hydrobiological point of view, the importance of this area is mainly due to the occurrence of a wide variety of aquatic habitats, supporting a number of valuable biotic communities. As a result of special biodiversity value of habitat, species and genetic level this area is among the most attractive scientific and protected site. The centre of the system is the ectogenic, crenogenic meromictic Lake of Köyceğiz. The lake fed by a series ground water and surface water inflows. The streams are the important sources that feed the lake. The benthic macroinvertebrate fauna of running waters which is mainly composed of aquatic insects (the aquatic insects represented by 93 genera among 118 genera of benthic macroinvertebrates) have been identified for future biological monitoring.

In this research, distribution of aquatic insects and other benthic macroinvertebrates of the running waters has been evaluated with physico-chemical parameters. The numerical analyses and a biotic index have been used as biological criteria for the assessment of water quality.

11-101

AQUATIC MACROPHYTES AND INVERTEBRATES IN NATURAL CULICIDAE BREEDING SITES OF BARINAS STATE, VENEZUELA.
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We describe changes in the invertebrate fauna from **Anopheles** breeding sites in relation to the aquatic macrophytes and precipitation regime in permanent and temporary pools in a area in which malaria is refractory in western Venezuela. As methodology we used the index of biotic integrity (IBI) (Karr, 1986) and the hypothesis of Habitat Selection (Buchwald, 1989). The data were first computed using a principal components analysis. Ordination of the points indicated changes in invertebrate fauna assemblage from permanent pools and separate from temporary pools. Depending of environmental factors, we found association with aquatic macrophytes and **Anopheles** larvae. Finally, we compared IBI with chemical variables to illustrate how this methodology can be applied for quantify associations factors. This IBI, based on 19 taxa attributes, showed close agreement with the precipitation and validated the hypothesis of habitat selection for **Anopheles** spp. in the study area.

This work received economical support from TDR/OMS Project ID880346.

12-001

Section 12

Ethology

12-002

CALLING AND HEARING PLASTICITY IN THE ENSIFERAN ORTHOPTERA.

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Most hearing systems have their threshold sensitivities close to the centre or predominant frequency of the signal used to bring the sexes together: the call frequency matches the sensitivity of the ear. There is increasing evidence of hearing mis-match between sensitivity and call carrier frequency in a range of acoustic insects including the ensiferan Orthoptera. When the predominant frequency of the song and hearing sensitivity are not aligned, or even where there is sexual dimorphism in both hearing structure and the physiology of the receptors in response to sexually distinct calls, the question is raised as to which selective processes produce these changes. The paper briefly reviews auditory mismatching in the ensiferan Orthoptera and focuses on two examples of hearing plasticity in West Australian bushcrickets. First, from the genus *Kawanaphilla* (Zaprochilinae), which has three species showing a range of hearing styles, from males with highly reduced auditory spiracles, rendering them almost deaf and females showing typical ensiferan sensitivity (*K. narree* and *K. mirla*), to a species that shows normal hearing sensitivities in both male and female (*K. yarraga*). We have gathered sufficient behavioural and ecological data to suggest that these systems may have evolved through sexual selection in response to different mating strategies. A second case concerns a sagine tettigoniid, *Scirasaga quadrata*, where sensitivities have thresholds at moderately high frequencies, between 15 - 20 kHz, while the song of the male is at the remarkably low frequency of 5 - 7 kHz. This species is subject to intense predation through an ormine parasitoid fly and its calling and mating strategy may have evolved in part through avoidance of predation.

12-001

FUNCTION IN PHYSICAL PARAMETERS OF ORTHOPTERAN SOUND SIGNALS

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The sound signals of acoustic Orthoptera include calling, courtship and rivalry songs. And the physical structure of these sounds is characterized by parameters of power, carrier and amplitude modulation pattern (AMP). A subset of these physical parameters is effective in communicating with potential mates and rivals about a sender's location, identity, quality and motivation. And signal morphology has also been shaped by interference from other environmental sounds and in response to eavesdropping exploitation by predators and parasites.

Signal parameter functions are made apparent by their underlying neurophysiology as well as the context in which they occur. Hypotheses of function can be tested by comparing signal parameters between related species, by whether they predict sender and receiver behaviour and by bioassay. This paper is a review of the evidence of adaptiveness in orthopteran signals from the perspective of the physical parameters that comprise the signal.

Power and carrier frequency are important in communicating a sender's quality to potential mates and rivals. But intensity is sometimes subordinated to pattern as animals establish identity. Signal power has been constrained in katydids by eavesdropping predators and interference has affected both carrier and pattern. For crickets, sinusoidal carriers over a limited spectral range provide accurate bearings to a distant male, while in grasshoppers and katydids broad band carriers may function to localize a sender by signal degradation. Calling song patterns, rather than power or carrier frequency, are the principal basis of encoding species identity and distinctive AMPs within the same song can be receiver-specific.

12-003

EVOLUTION OF CHORUSING: PRECEDENCE, RELATIVITY, AND SELECTIVE ATTENTION

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The synchronous and alternating choruses produced by males in many acoustic species are epiphenomena resulting from individuals competing to jam each other's signals. This arises when females disregard all but the leading signal in a sequence, and males who lead are selectively favored. Given this perceptual bias, modeling shows that a resetting of signal rhythm, which generates either synchrony or alternation, is evolutionarily stable provided that resetting includes a relativity adjustment for the velocity of signal transmission and selective attention toward only a subset of competitors. Signaling strategies in chorusing insects and anurans incorporate these predicted features.

12-004

ACOUSTIC SIGNALLING AND CONSTRAINTS ON SEXUAL SELECTION IN SAGEBRUSH CRICKETS (HAGLIDAE).
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Sagebrush crickets (*Cyphoderris strepitans*) breed during early spring in the mountainous regions of western North America. Each evening during the 4 - 6 week mating season, males emerge from the soil litter, climb sagebrush, and call for several hours. Males are able to call at remarkably cold temperatures, as low as -8°C. During copulation, females feed on the male's fleshy hindwings and the resultant flow of hemolymph. As a consequence, it is possible to determine male mating status in the field by direct examination of the hindwings. Further, male lifetime mating success can be determined in the field by mark-recapture and sequential examination of the hindwings of recaptured individuals.
Field and laboratory studies have shown that in this species calling functions both in the attraction of females and in male spacing behaviour. Calling, per se, is important to male mating success; males rendered mute do not get mated. Males are not territorial, but spacing behaviour appears to be mediated through acoustic signalling and the mutual avoidance of rivals.
A two year field study revealed that male lifetime mating success is related to adult longevity and the number of nights during the breeding season spent calling. Most males mated only once, or not at all. The opportunity for sexual selection was low, and differed between years. Virgin males obtained matings at a frequency higher than predicted by a random mating model. This virgin male mating advantage appears to constrain the opportunity for sexual selection in the species.
The mating system of sagebrush crickets resembles that of some anurans. There is an explosive early spring breeding season, intense inter-male competition, and chorus tenure is the major predictor of male mating success. Unlike most anurans however, the opportunity for sexual selection in sagebrush crickets is rather low.

12-006

THE "SHAPE" OF FEMALE MATING PREFERENCES, AND WHAT THIS TELLS US ABOUT THEIR EVOLUTION.

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The "shape" of female mating preferences (or the "preference function") is an important component of models of sexual selection and communication. Whether preferences are directional or stabilising can influence the dynamics of the communication system. The extent of overlap between the shape of preferences and the current distribution of male traits will determine the strength of sexual selection on male traits and indicates the extent to which coevolution, species recognition or arbitrary sensory biases may have influenced the evolution of the communication system. Orthoptera, especially crickets, provide an ideal model system for studying the variation and genetics of this trait. I review how much progress has been made in measuring this, and present new results using a technique developed to measure variability in female preference in the bushcricket *Ephippiger*. Preliminary results suggest that the shape of preferences varies with different structural components of the signal. There is evidence that preferences are genetic and can vary between individuals. Even within *Ephippiger*, some preferences imply coevolution, others "sensory drive", and others are primarily exerting sexual selection.

12-005

TEGMEN ASYMMETRY AND ITS INFLUENCE ON FREQUENCY MODULATION AND SONG PREFERENCES OF FIELD CRICKETS (*GRYLLUS CAMPESTRIS* L.)

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Acoustic signals produced by males are the traits most often subject to sexual selection. The mechanism of song production in crickets is well documented. However, contrary to previous work, I show that cricket songs exhibit frequency modulation to varying degrees, dependent on the degree of asymmetry in the sound resonators or harps. Recent studies of sexually selected traits suggest that size and symmetry may reflect male quality. The harps of crickets exhibit directional asymmetry yet show patterns similar to those of traits that exhibit fluctuating asymmetry; large males have large and relatively symmetrical harps. Females prefer pure tones of low carrier frequency, characteristic of large symmetrical harps. However, small males can partially compensate for their high carrier frequency by producing frequency modulated song. Directional asymmetry in harps may arise because of a selection bias favouring an alternative signalling strategy among males of low quality.

12-007

CHORTHIPPUS PARALLELUS: WHAT DOES IT TELL US ABOUT MATING SIGNAL DIVERGENCE?

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The mating behaviour, including both acoustic and chemical signals and mating preferences, of the grasshopper *Chorthippus parallelus* (Orthoptera: Acrididae) has been studied both in a hybrid zone between parapatric subspecies and more broadly around Europe. Originally focused on the possibility of reinforcement of prezygotic isolation in the hybrid zone, this project has now moved on to consider the interactions between the two signalling channels, the likely processes underlying their divergence, and the selection pressures operating on them. Displacement of clines in the hybrid zone provides the opportunity to study a wide range of signal combinations while background information on the current distribution and colonisation pattern of the grasshopper in Europe allow tests of competing hypotheses about divergence.

12-008

THE BEHAVIORAL ECOLOGY OF FEMALE CHOICE IN TREE CRICKETS

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Although evidence of mate choice is now well founded, distinguishing among alternative evolutionary models of female choice remains problematic. An approach that has been surprisingly under-utilized is the study of the functional design of preferences. My goal during this talk is to make two points about the value of studying preference design for understanding sexual selection. First, models of sexual selection make fundamentally different predictions about the way in which preferences should be structured. Thus studying the structure of preferences provides a functional (teleonomic) approach to testing alternative models of sexual selection. To illustrate this point, I present work on female preferences in tree crickets that contrasts predictions of indicator models with those based on runaway sexual selection. Indicator models predict that choices should be (1) based on comparisons, (2) directed at elements of sex-limited signals that indicate fecundity benefits and/or heritable benefits, and (3) that these elements should lack susceptibility to cheating. In comparison, runaway models predict that choices are (1) fixed within females and (2) show consistent variability among females. Results of experiments on female choice for song showed that comparisons were necessary for choices, females preferred low-frequency songs, and low frequency was a reliable indicator of both fecundity and putative heritable benefits of mating, supporting all 3 predictions of indicator models. By contrast, female preferences were neither fixed within nor variable among females, falsifying the necessary conditions for runaway selection. My second point is that understanding the design of preference can also elucidate social and environmental factors influencing the direction and intensity of sexual selection. To illustrate this second point, I present a study that shows that both the relative coyness of females and the degree to which they differentially mate with larger males depends on the benefits of mating, which in turn depends on food quality. Thus I predict that the intensity of sexual selection and possibly even the criteria of female choice will vary with the environment.

12-009

THE FUNCTION OF LENGTHY COPULATIONS IN A BUSH CRICKET

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Copulation duration varies dramatically in insects, ranging from a few seconds in certain chironomid flies to several days in some butterflies and bugs. Variation in copulation duration can be related to sperm transfer (including manipulation of previous males' ejaculates), mate guarding and/or mate assessment. Males of the Australian bushcricket *Coptaspis sp 2* mate for several hours. Unlike most bushcricket species, males of this species remain attached to the female for up to 6 hours after transfer of the sperm containing ampulla, which he attaches externally to the female's genitalia. Females mate multiply and males are capable of re-mating soon after a copulation has ended. The function of these lengthy copulations was examined experimentally. The number of sperm present in the ampulla in relation to varying copulation durations was determined, and the influence of female mating status on the number of sperm transferred and copulation duration was assessed. Finally, male fertilization success in relation to copulation duration was examined in an attempt to determine the main function of these lengthy copulations.

12-010

THE BUSHCRICKET SPERMATOPHORE: A FEMALE PERSPECTIVE

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During mating, many male bushcrickets transfer a large gelatinous mass, the spermatophylax, attached to the sperm containing ampulla. Immediately after mating, the female consumes first the spermatophylax and thereafter starts to eat the ampulla. Concerning the function of the spermatophylax, there are a number of recent papers discussing whether this nuptial gift represents male mating effort or male parental investment. In many species, the spermatophylax seems to be exactly the size to secure sperm transfer from the ampulla to the female spermatheca. Since the duration of this process can be influenced by the female - at least on an evolutionary time scale -, the female should control also the size of the spermatophore. Little attention, however, has been paid to the question which factors should influence the female "decision". Possible advantages the female might receive by eating a large spermatophore have rarely been discussed or measured, apart from effects of increasing egg number or size after mating. By examining the behaviour of females of *Poecilimon* species which have a relatively high mating frequency (mating every day or every second day) I try to understand and quantify the direct benefits for the female. Spermatophore size per se gives only limited information. More important aspects are (1) the period of time the female does not need to look for food after receiving a spermatophore, and (2) reduced predation pressure during this period due to reduced motility and mortality. To know how spermatophylax size affects female survival may be important in order to understand the evolution of spermatophylax size.

12-011

THE ENERGETICS OF MALE MATING BEHAVIORS IN THE HOUSE CRICKET, *ACHETA DOMESTICUS* L. (ORTHOPTERA: GRYLLIDAE), AND THEIR IMPLICATIONS FOR ALTERNATIVE MATING STRATEGIES IN THE GRYLLINAE

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Knowledge of the costs of alternative behaviors is critical to understanding when and why a particular behavior is observed. Flow-through respirometry of male house crickets revealed that their rates of oxygen consumption during contests over burrows increased by 5-8 times resting rates. During terrestrial locomotion, i.e. walking or running, males consumed oxygen at 3-5 times resting rates. In contrast, acoustic advertisement for mates raised male oxygen consumption by only 10-30% above resting rates, or an order of magnitude less than either combat or locomotion. Similar relative energetic costs for these behaviors in other crickets may explain the appearance of alternative mating strategies with changes in competitor density and adult sex ratio.

12-012

LONGITUDINAL STUDIES OF SELECTION ON THE TEXAS FIELD CRICKET: THE SIGNIFICANCE OF PARASITOID FLIES.
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Acoustic signalling in *Gryllus integer* is important in attracting conspecific females for mating. Callers, however, also attract acoustically-orienting females of the tachinid parasitoid, *Ormia ochracea*. Fly females larviposit on and around males and infested males die in 7-10 days. Males are under opposing selective pressures; calling is important for reproductive success, but may result in premature death. Fly numbers, however, vary seasonally and so selection pressure from *Ormia* may change across the year. Longitudinal studies were carried out to examine the relationships between calling activity, searching, mating success and longevity. Individually marked groups of 10 male and 10 female *G. integer* were released into large 30m² outdoor arenas and observed for 2-3 weeks. The arenas were covered by fine netting to control fly access to the experimental animals. Gravid *Ormia* females, captured locally, were used in some of the arenas and not in others. Replicates were carried out both in the presence and absence of the ormine parasitoid. Flies were found to be phonotactically active throughout the scotophase except when the temperature dropped below 12 °C. Flies within the arenas oriented to calling song but also, to a lesser degree, to courtship and aggressive stridulation. Infestation rates in the fly-present arenas was high; 40 -70% for males and 24-30% for females. Females were infected as a consequence of responding to male calls. Parasitized males had significantly longer mean nightly calling durations than unparasitized males, suggesting that prolonged calling activity carries a heavy cost in terms of attracting parasitoid flies. Increased calling activity was, however, positively correlated with relative male mating success. Selection gradients and selection differentials were calculated and indicated significant positive selection for calling in *G. integer* males, even with flies present.

12-014

CALLING BEHAVIOUR, AGE AND SURVIVAL IN BUSHCRICKETS: THE ROLE OF SOUND LOCATING PARASITIC FLIES?

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During the summer months in south-western Australia adult males of the univoltine bushcricket *Sciarasaga quadrata* (Tettigoniidae: Austrosaginae) call to attract mates. However, calling males also attract the acoustically orienting parasitic fly *Homotrixa alleni* (Tachinidae: Ormiini). Parasitised male *S. quadrata* will only live for a further 2 weeks whereas unparasitised males live and continue to call for up to 4 months. Parasitism rates among surviving males steadily increase during the calling season and may be as high as 87% by the season's end. An experiment was conducted to compare the calling behaviour of males that had escaped parasitism throughout the entire calling season to that of all males at the season's beginning (prior to the commencement of parasitism) and to that of surviving males at intervals throughout the calling season. Males were also tested for age related changes in calling behaviour. Calling parameters measured included the onset, duration and temporal pattern of calling. Results are discussed in relation to estimated risks of parasitism and the flight activity periods of gravid female *H. alleni*.

12-013

PHONOTACTIC PARASITIDS AND CALLING BEHAVIOR IN THE FIELD CRICKET *TELEOGRYLLUS OCEANICUS*
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Acoustically-orienting parasitoids take advantage of male mating advertisement calls to locate hosts, putting the male simultaneously at risk of being parasitized by calling and of losing potential mates by remaining silent. A particularly intriguing example of such a host-parasitoid relationship is seen in the field cricket *Teleogryllus oceanicus*, an Australasian species introduced to Hawaii. In Hawaii, but nowhere else, it is subject to parasitization by the phonotactic parasitoid fly *Ormia ochracea* (Tachinidae: Ormiini). Other studies of such cricket-fly systems have suggested the existence of a silent satellite class of males, presumed to avoid detection by the parasitoid and intercept females as they move toward calling males. In contrast, noncalling *T. oceanicus* males occurred with callers in all locations, and silent males were actually more heavily parasitized than callers. Parasitized populations of crickets from several locations in Hawaii differed from non-parasitized populations from Australia and the Pacific in call structure, the diel distribution of calling, and the sex-specific age structure. Dawn and dusk calling is curtailed in parasitized populations, and males from Hawaii are younger than males from uninfected populations. Variation in parasite prevalence helps explain variation in temporal structure of the calling song, although other factors also influence this variable. This system presents an excellent opportunity to examine conflicts between natural and sexual selection.

12-015

A MATING HISTORY OF KATYDIDS, CRICKETS AND WETA (ENSIFERA)
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I use recent phylogenies of the Ensifera to trace the evolutionary history of reproductive behaviours that typify many taxa in this orthopteran suborder. These include, calling behaviour by males that precedes pair-formation in many taxa, the mating position, mate feeding by males (on glandular secretions, body parts or nutritious spermatophores) and maternal care of eggs and/or nymphs. Some phylogenies support the hypothesis that male tegminal (and tibial ears) evolved more than once.

12-016

SEXUAL SELECTION AND SPERM COMPETITION
A. Parker (Liverpool-United Kingdom)

ABSTRACT NOT RECEIVED

12-017

A MOLECULAR BASIS FOR SPERM COMPETITION IN *DROSOPHILA* *MELANOGASTER*

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Sperm competition in *Drosophila melanogaster* has been investigated.

In one study, the hypothesis that males are passing a "spermicide" substance(s) to remating females was tested using interrupted matings and a transgenic line which results in ablation of the accessory gland. Evidence is presented that males pass a substance(s) from the accessory gland that incapacitates or dislodges stored sperm.

In a second study, genetic variation for sperm competition parameters was measured in relationship to accessory gland gene allelic variation. The allelic variation was almost entirely associated with first-male resistance to sperm displacement.

12-018

PLASTICITY IN MALE REPRODUCTIVE STRATEGIES AS ADAPTIVE RESPONSES TO VARIABLE RISKS OF SPERM COMPETITION

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Reproductive environments are variable but often predictable. Because of this variability, individuals may be selected to be plastic in the strategy they adopt for optimal reproduction. An important factor which determines mating pattern is population structure: at higher densities females may mate more frequently, generating higher risks of sperm competition for males. Males should therefore be selected to be sensitive to population structure in order to tailor a strategy which delivers the greatest reproductive success.

In the semelparous meal moth *Plodia interpunctella* (Lepidoptera: Pyralidae), all resources for the brief period of reproduction are accrued during the larval development stage. Population structure is variable and female mating pattern is positively related to population density. Males therefore emerging into dense populations encounter higher risks of sperm competition. By manipulating larval population structure, but maintaining food levels per capita constant, I demonstrate that developing male *P. interpunctella* are sensitive to population density and tailor a reproductive strategy to fit the predicted adult reproductive environment. I measured the following constituents of a male reproductive strategy: development time, relative gonadal investment, ejaculate sperm numbers, relative head/thorax and abdomen size, and adult longevity. Males from high densities anticipate frequent mate encounter and elevated risks of sperm competition and appear to invest in a strategy for mating and sperm competition competence. At lower densities males anticipate rare mate encounter and low risks of sperm competition, and appear to invest in a strategy of migration and mate searching. Such flexible male responses represent a 'reaction norm' where male strategies vary as a continuous function of the predicted reproductive environment's signal.

12-019

SPERM COMPETITION IN THE BUSHCRICKET *POECILIMON* *HOELZELI* (ORTHOPTERA, TETTIGONIOIDEA) IN RELATION TO THE NUMBER OF INSEMINATING MALES

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Most studies on sperm competition in insects focused for methodological reasons on a situation, in which the sperm of only two males compete for the fertilization of a single female's eggs. In the majority of these cases the second mate gains most fertilizations. In many insect species, however, more than two matings of a female are common in the field. We investigated the outcome of sperm competition in multiple mated females (2 to 5 matings) of the bushcricket *P. hoelzeli*. The paternity of the progeny was determined by DNA microsatellite analysis.

There was no change in the sperm precedence pattern with respect to the number of mating partners of a female. On average the last mate predominated at fertilization. The proportion of offspring sired by the last mating male varied over an extremely wide range (mean = 63%, range = 100% - 0%). It was dependent on male age at copulation and male size. The success of an individual male increased with age at copulation and with body size.

The present study demonstrates that even in a polyandric mating sequence the position of a copulation is important in determining sperm precedence. However, individual variation in the proportion of offspring sired by the last mating male may influence differential reproductive success among males.

12-020

GENITAL MORPHOLOGY, SPERM COMPETITION AND SEXUAL SELECTION IN *ALEOCHARA* SPECIES (COLEOPTERA: STAPHYLINIDAE)

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During copulation males of the rove beetle *A.curtula* (Goeze) introduce a flagellum of the endophallus as a guidance for the tube of the spermatophore into the female's spermathecal duct. After separation of the pair, the tube further elongates and passes through a valve at the basis of the sclerotized, U-shaped spermatheca. Here the tube swells to form a balloon filling most of the spermatheca. Sperm of previous copulations are backflushed, indicative of last-male precedence, which could also be established by DNA-fingerprinting. The balloon of the spermatophore is pierced within the spermatheca by tooth like structures releasing large number of small sperm.
Other species of the genus exhibit a whole variety of complicated male and female genital structures. In *A.bilineata*, the spermatheca with two valves forms narrow coilings at its base. The spermatophore tube opens just before, and free sperm of significantly larger size enters the spermatheca. In *A.tristis*, the spermathecal duct is spiral and has double the length of the female's body. Correspondingly, the male's flagellum forms a spiral and is introduced into the spermathecal duct. Here also free sperm of large size enter the spermatheca.
Function and morphology of *Aleochara* genitalia, spermatophore and sperm are discussed in the view of sperm competition and female choice. Additionally, the mating strategies have been investigated behaviorally and ecologically with respect to polygamy, male territoriality, female coiness, mate guarding, pheromonal communication, and sperm precedence by using molecular techniques.

12-022

MATECHOICE AND SPERM TRANSFER IN THE AUSTRALIAN SHEEP BLOWFLY *LUCILIA CUPRINA* (DIPTERA: CALLIPHORIDAE)

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Female *L.cuprina* contribute significantly to the production and survival of offspring, whereas males provide no investment in their offspring beyond their sperm. Sexual selection theory predicts that this asymmetry would favour mate choice by females, however evidence of mate choice by females is very limited, with females only appearing to be less willing to mate with previously mated males. Males discriminate against non-virgin females smaller than themselves. As the size of the male increases relative to the size of the female, the male takes longer to make the first mating attempt.

In this study, large and small adults of each sex were paired in four different combinations to look for evidence of mate choice both prior to and during mating. Males were placed into standard mating containers and once the female was introduced, a record was taken of the time from pairing to the first mating attempt, the number of mating attempts and copula duration. The numbers of sperm transferred to small and large females were estimated from spermathecal squashes under a millimetre grid. In addition, large or small females mated by either small or large males were allowed to oviposit freely until death to determine lifetime reproductive success. The fecundity of *L.cuprina* females is strongly and positively correlated with size, so by choosing to mate with females larger than themselves, males would be expected to increase their own reproductive success. The results of this study are discussed in the context of sexual selection and mate choice theory.

12-021

PRE-AND POST MATING FEMALE CHOICE IN GRASSHOPPERS (ORTHOPTERA: ACRIDIDAE)
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Grasshopper mating systems have not received as much attention as those of their close relatives, the katydids and crickets. The relative role of pre and post-mating female choice in shaping the mating behavior of three species of grasshoppers, *Chortophaga viridifasciata* (De Geer), *Dichromorpha viridis* (Scudder), and *Schistocerca americana* (Drury) was examined. In general, females encountered and so chose among males sequentially. Species varied in the degree of pre versus post mating choice exhibited by females. In all species, even in *C.viridifasciata* where females typically exhibited strong mating preferences, there was evidence that females did also choose among males more cryptically by controlling the timing of a second mating, copulation duration or how sperm was stored. Some of the mitigating factors, such as a male's relative ability to deliver nutrients, have also been identified.

12-023

LEK BEHAVIOR OF FRUIT FLIES

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Lek behavior is a relatively common mating system in drosophilid and tephritid fruit flies. Still, detailed quantitative studies have been conducted for only a few species. Here, we focus on the Mediterranean fruit fly (*Ceratitidis capitata*), the best studied species, to examine key questions common to lek-forming species in general.

Several laboratory studies have demonstrated nonrandom mating among males, with a small subset of males accounting for a disproportionately large number of matings. Identifying the factors responsible for this trend is difficult, and only overall indicators of sexual activity were correlated with mating frequency. Male size and fighting success appear unimportant. Recent work has shown that parapheromones may affect male mating success: males given access to trimedlure or a-copaene achieved more matings than unexposed, control males. Despite the apparent operation of female choice, tests regarding the adaptive benefits of female choice were inconclusive. No significant relationship was found in father-son mating success, and data on direct benefits (enhanced fecundity) yielded weak trends, which were difficult to interpret.

In addition to examining mate choice within leks, we will also evaluate hypotheses relating to the evolution of lek behavior, with particular emphasis on the importance of predation on signaling males. The role of male "quality" and number and food resources in affecting female arrivals at leks (hotspot model) will also be addressed.

12-024

MATING SYSTEMS OF SONORAN DESERT DROSOPHILA (DIPTERA:DROSOPHILIDAE)

T.A.Markow, C.M.Breitmeyer

Our ability to evaluate the interplay between phylogeny and recent ecological adaptations in mating system variation is often restricted by limited information on either the systematic or ecological relationships of a specific group of organisms. Phylogenetic relationships of flies of the genus *Drosophila* have been well characterized. Four species of *Drosophila* are endemic to the Sonoran Desert of North America and utilize necroses of specific host cacti to feed and breed. We have found that these cactus species differ significantly in resource availability on both temporal and spatial scales. The four fly species exhibit enormous diversity in their mating system features, raising the question as to whether this variation reflects their long-term evolutionary history or their resource ecology. We employ our expanding ecological data for these *Drosophila* species to examine the influence of resource availability on patterns of allocation to reproduction.

12-025

MATING IN FEMALE *DROSOPHILA MELANOGASTER*: COSTS, BENEFITS AND THE EVOLUTION OF MATING FREQUENCY.

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Female *Drosophila melanogaster* from both laboratory stocks and field populations can show high frequencies of remating. Why females should mate multiply is the subject of several adaptive explanations and is influenced by a number of environmental factors. Female mating frequency is heritable and shows a bi-directional response to artificial selection, suggesting an evolutionary history of stabilising selection. Too low a frequency of mating may be non-adaptive because females run the risk of running out of sperm and too high a mating frequency is also costly. The cost of mating is caused by the transfer of non-sperm components of male seminal fluid to females at mating, and may represent a side-effect of evolutionary conflict between males. The manipulation of female life histories by these male 'accessory gland' components may have implications in the control of insect pests.

12-026

PATTERNS OF HOST USE BY FEMALE INSECTS AND THEIR SIGNIFICANCE FOR INTERACTIONS BETWEEN THE SEXES

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Studies of resource defense mating systems to date have generally failed to capture the complexity of resource utilization by females and its consequences for interactions between the sexes. Likewise, the substantial literature on the dynamics of foraging behavior by female insects has largely overlooked the role of males in shaping those dynamics.

We describe an unusual host-defense mating system in walnut-infesting flies in the genus *Rhagoletis*. In two such species native to southern Arizona, *R. juglandis* and *R. boycei*, females show an unusual propensity to add eggs to previously-established egg-laying cavities. Males defend fruit territories and in particular guard egg-laying cavities. The function of the female's behavior remains at issue; by contrast, site preference on the part of the male is associated with clear improvements in male mating success.

Other instances in which male preference in terms of resource defense mirrors female preference in terms of egg-laying are presented. In each case, male preference is expressed even in the absence of females. Finally, ways in which host use is dynamic over the life of individual females are enumerated and early evidence that male behavior is dynamic in parallel fashion presented. We end by discussing how male tracking of female resource preference might itself affect that preference, sometimes in unexpected ways.

12-027

PHYSIOLOGICALLY INDUCED CHANGES IN THE MATING BEHAVIOUR OF TSETSE FLIES

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The mode of reproduction of tsetse flies, by adenotrophic viviparity, is unusual among the Diptera and is associated with many unique aspects of the tsetse's mating system. The behavioural repertoire associated with mate location and identification, courtship and copulation is regulated by external physical and chemical stimuli as well as by internal physiological mechanisms. This paper will discuss research designed to elucidate the mating behaviour of tsetse and its regulatory mechanisms.

12-028

MALE DISTRIBUTION AND MATE SEARCHING SUCCESS AT PATCHILY DISTRIBUTED RESOURCES

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I will show results on male mate searching success in two fly species where males gather at female oviposition sites. Using the ideal free distribution as basic model, male distribution at oviposition sites and male gain rates in terms of mating success are examined. In both species, short term mating success was positively related to male size. However, even within large males there were differences in movements and mating success. This suggests the importance of identifying individuals and their differences and not assuming, for example, that males with the same phenotypic character have equal gain rates.

12-030

SWARMING IN EMPIDID FLIES

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Dance flies are predacious insects which often form male mating swarms and in many species males collect an insect prey item prior to swarming, which is presented the female at copulation.

In the species *Empis borealis*, females, however, gather to swarm and males carrying prey visit swarms for mating. Males either mated with one of the females (accepted swarms) or left swarms (refused swarms) without mating. Males mated with younger (low wing wear) and relatively larger (wing length) females. Males stayed shorter as females size variation in swarms increased and males more often left smaller than larger swarms without mating. Female mating status (virgin/non-virgin) or proximity to oviposition did not influence her likelihood of copulation. Male visiting rate in swarms and mating rate per female increased with swarm size. We suggest that *E. borealis* males discriminate among females, but found no evidence for male competition or female choice.

The mean swarm size was 4.85 ± 4.54 females (median 4.03). The very same swarm site was used for several years and some swarm sites held larger amount of females than others. Larger experimental swarm-markers attracted both more males and females. The number of females attending a given swarm site is also supposed to be influenced by male arrival pattern, male preference for larger swarms, the inability of males to judge the absolute body size of females, and female polyandry.

Females are polyandrous and females containing more sperm also had more developed eggs. The nuptial gift nutrients provided by the male seem to contribute to egg production and possibly limit female reproductive success, giving rise to the sex role reversed mating system in *E. borealis*.

12-029

COSTS OF SWARMING BY ANOPHELINE MOSQUITOES

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A cost-benefit analysis of the swarming behavior of male *Anopheles freeborni* in California and *A. sergentii* in Israel will be presented.

The activity of predators affects the timing of copulations in swarms. Dragonflies attack swarms of *A. freeborni* during the early part of the swarming period, and most copulations take place after attacks cease. Conversely, swarms of *A. sergentii* are attacked by insectivorous bats which appear late during the swarming period. In this system most copulations occur before the predators appear. Glucose and glycogen fuel swarming flight. On average the amount of energy used is more than 50% of the reserves available to males- thus success at sugar feeding is linked to reproductive success.

The costs of swarming are energy expenditure and risk of predation, while the obvious benefit is reproductive success. For *A. freeborni* this benefit is not shared equally by all investors- large males copulate significantly more frequently than small ones. Size appears to be the cardinal factor determining initiation of swarming for males. Smaller males are relegated to commence swarming earlier, when predators are active and females relatively scarce.

12-031

ACOUSTIC COMMUNICATION IN THE AUSTRALIAN WHISTLING MOTHS - A CASE OF SENSORY EXPLOITATION.

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It may be assumed that the ears of moths have evolved primarily to detect the presence of bats and are normally tuned to sonar frequencies most used by bats in prey detection. The behaviour of the moth is usually predictable and, in the presence of bats, the moth will take evasive action. There are a number of cases within the Lepidoptera where sound is used either for aposematic display or sexual attraction and, in the later case, we may presume that ears evolved for prey detection will now be used to detect the social signal. In this case the nervous system must resolve an apparent conflict between the historical situation of prey avoidance and the more recently evolved condition of mate attraction. One solution is for the moth to fly in the absence of bats, either within secluded habitats or during the day when bats are not active.

Two species of whistling moth, *Hecatesia exultans* and *H. thyridion* (Agarastinae: Noctuidae) inhabit different regions of the coastal areas of Western Australia and males of both species use an acoustic display to defend territories and attract females. One species is day flying, and calls from perches within defended territories, while the second calls in the evening and uses an areal acoustic display. This paper examines the hearing sensitivities of both species of moth, their calling and mating strategies in the field, and focuses on the shift in roles between predator detection and avoidance to mate attraction.

12-032

SUBSTRATE TRANSMITTED SIGNALS IN THE
BROWN PLANTHOPPER, *NILAPARVATA LUGENS*
(HOMOPTERA: DELPHACIDAE)

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Brown planthopper males signal to females using vibrations transmitted through plant stems. The pulse repetition frequency of the male signal is known to vary geographically and between host associated races. Here we report on structural complexity in the signals that has not previously been described, on the pattern of female preference for features of the male signal and on correlations between male signals and mating success. There is evidence for sexual selection favouring high pulse repetition frequency, although females respond to a wide range of signals. Pulse frequency modulation has less effect on female response or male mating success. The results are discussed in relation to divergence in signals among populations.

12-034

INTEGRATION OF VISUAL AND CHEMICAL SIGNALS IN
MATE-FINDING OF MOTHS

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The mate-finding behaviour of moths generally involves an upwind flight response of a male to the volatile sex pheromone of a conspecific female. This response of male moths has been the subject of much work, and is thought to involve the behavioural mechanism of optomotor anemotaxis, the use of visual information to steer a resultant track and groundspeed with respect to the wind, as well as modulation of certain manoeuvres through contact with the fine structure of the pheromone plume. Thus, a male moth continually integrates appropriate chemical and visual stimuli as it progresses upwind.

We have studied the behaviour of flying male lightbrown apple moths, *Epiphyas postvittana* (Walker) as they approach and land on or near pheromone sources. Where the male lands is influenced strongly by both chemical and visual stimuli. The pheromonal stimuli are important because the male must maintain contact with the plume as they influence the manoeuvres of the male, which subsequently affects where he lands. For example, physical objects within the plume create turbulence, thereby changing the fine structure of the plume, and affecting where the male lands. Visual stimuli influence the manoeuvres of male moths. If these stimuli are imbalanced, with respect to the axis of the wind, the male's flight tracks are skewed towards the greater visual stimulus; with a choice of sources, the male tends to fly towards the source(s) on the side with the greater visual stimulus. The landing behaviour, and especially where the male lands with respect to the pheromone source, is also influenced strongly by visual stimuli. These stimuli are, we believe, related to those used by the male during optomotor anemotaxis, rather than any specific visual stimulus relating to the female moth.

12-033

FRUIT FLY PHEROMONES AND FRUIT TREES

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While participating in leks, male Caribbean fruit flies, *Anastrepha suspensa* (Loew), apply everted anal membranes associated with pheromone glands to the surface of leaf-territories. This dipping coincides with periods of wing fanning which may disperse pheromones in addition to generating an attractive acoustic signal. Some pheromone components remain on the leaf for at least 1 hour. Females are attracted to leaves that have been previously occupied by mature males, presumably because of the chemical cues deposited on their surfaces. The value of a territory may be increased by the accumulation of pheromones and this might contribute to aggression among males and the defense of certain leaves. Pheromone production, the relationship of pheromones to the chemicals present in hosts and possible cases of deposition on substrates in the Tephritidae are reviewed. Advantages of placing pheromones and other attractive signals on objects, such as increasing emission surfaces and misdirecting predators, are considered in a variety of insects.

12-035

MATE LOCATION BY PENTATOMIDS MEDIATED BY
SUBSTRATE-BORNE SIGNALS

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Pentatomide landbugs emit sound signals by tymbal located at the dorsal side of abdomen. The dominant frequency of their signal emissions lies between 80 and 150 Hz. Males and females of the same species emit songs with similar frequency but significantly different time characteristics. Species specific sound emissions are characteristic for pentatomids; differences are more pronounced in males than in females. Still recent investigations have shown that time parameters of the male and female songs of *Nezara viridula* from different continents are characteristically different. Sound communication in the form of calling, courtship and rival songs plays a vital role in premating behaviour enabling among others also mate location and species recognition. Small diameter of tymbal and low frequency of the emitted signals prevent their effective transmissions via air. Laser vibrometry of songs transmitted as substrate-borne signals as well as behavioural experiments on their host plants have shown that communication via substrate might be effectively performed at distances of at least 1m. Lower frequency components are transmitted on the plant at longer distances than the higher frequency parts. The subgenual organ with just two scolopidia is tuned to frequency characteristics of pentatomide sounds. Leg campaniform sensilla and joint chordotonal organs together with well developed Johnston's organ and campaniform sensilla on antennae might participate in reception of the low frequency parts of their songs transmitted as substrate-borne sound on their host plants.

12-036

LEKS OF PAPER-WASPS:
VISUAL AND CHEMICAL SIGNALS USED IN MATE ATTRACTION

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A lek is an aggregation of males displaying in a nuptial arena, where they occupy and defend contiguous territories lacking in resources useful to females (in the case of paper-wasps, a few branches of a tree, some stones, a bush, segments of fences or poles, more or less conspicuous landmarks, and so on). Male activity at these potential mate-encounter points, which are species-specific and basically stable in space and time, consists of struggles, pursuits, threats and self-advertisement displays, i.e. flights along fixed patrol routes, marking and attendance at exclusive perches.

Aggregations of flying wasps represent short-range visual cues for conspecifics of both sexes, whereas scent-marking might act as a relatively long-distance attractant and enhance the visual signal. Males of several species of *Polistes* rub the abdomen, face or legs on habitual perches, a behaviour commonly explained as a pheromonal release. On the other hand, *Stenogastrinae* males patrol and defend aerial territories from intruders, without marking or perching on the substrata. Males of this group perform a conspicuous 'stripes-display' by fully stretching the abdomen in flight; this movement reveals three large white stripes on the tergites (a sexually dimorphic trait) and probably elicits a glandular secretion from tegumental cells. Visual and chemical components of male displays in paper-wasps will be discussed in relation to mate attraction.

12-037

RELATIONSHIP BETWEEN VISUAL FIELD STRUCTURE AND
MATE-FINDING TACTICS USED BY MALE BUTTERFLIES

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Many male insects use a sit and wait tactic to locate females. This tactic places a number of conflicting demands on males that include (1) the need to be on the best perch and in the best body orientation to detect females, and (2) the need to maintain a body temperature permits a quick response to passing females. We have been studying the nature and resolution of these conflicting demands in the desert hackberry butterfly, *Asterocampa leilia*, in the Sonoran desert of North America. Males in this species occupy and defend perches on or adjacent to the larval foodplant where they sit and await the arrival of females. Males leave their perches to approach and chase females that fly by.

During the morning when males perch, they maintain a body temperature of about 38-40°C through adjustments in body orientation, wing position, and perch substrate preferences. They begin the morning perched on the ground and later switch perches about 1 m above the ground on the larval foodplant. Does this affect their ability to detect visually females passing nearby? Our data suggest that: (1) females fly through male perching areas at an altitude of about 1 m, (2) when perched on the plant the male's visual system is optimally positioned to detect passing females given the structure of the male visual system and his head orientation, and (3) when perched on the ground, males are in a less than optimal location for detecting females. We conclude that on cool mornings a compromise is struck between mate detection and thermoregulation that is in favor of maintaining body temperature in the preferred range.

12-038

MATE-FINDING IN COCKROACHES

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Male cockroaches have diverse orientation mechanisms for locating mates. They are able to employ spatial processing, whereby they compare the inputs to the two antennae in determining in which direction to move. They also employ temporal processing, whereby they sample female sex pheromone over time and use the simple rule of turning if the concentration goes down and moving straight if the concentration continues to increase. When the pheromone is in a wind current, male cockroaches can use zig-zag upwind orientation to locate the female. In addition, male cockroaches move downward when they detect female sex pheromone, assisting them in locating pheromone-secreting females that are below them.

12-039

SOUND COMMUNICATIONS OF TWO SPECIES OF PLEASING FUNGUS BEETLES *DACNE JAPONICA* AND *D. PICTA* (COLEOPTERA: EROTYLIDAE) USING TWO TYPES OF SOUND PRODUCING APPARATUS

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Dacne japonica and *D. picta* are pests of the Shiitake (*Lentinus edodes*), the most popular edible mushroom in Japan. Both species are often found aggregated on single mushroom. Males stridulated to chirp using vertex-pronotal apparatus in context of aggression and courtship. The numbers of syllables in chirp and the numbers of chirps in sequence differed significantly between these behaviors in both species. It seemed that there were also differences between species in these chirp characteristics.

Both sexes of both species had elytral binding-patches and at least *D. picta* females used these patches as elytra-abdominal apparatus to produce click sound that kept alerted individuals alert. This is the first record for sub-social beetles to produce a social-functional sound.

12-040

THE INFLUENCE OF A PARASITOID FLY ON SOUTHERN EUROPEAN BUSHCRICKETS

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One function of the bushcricket's song is to attract females of the same species for mating. Predators however, who detect their prey by means of acoustic orientation are also attracted. Among these the tachinid *Therobia leonidei* (Mesnil, 1964) (Diptera: Tachinidae) has a great influence on the males of several bushcricket species in Greece. Within one season the percent of parasitized animals in a population can rise to more than 50%. In the genus *Poecilimon* closely related species produce songs with different syllable numbers within each verse. In this study I want to measure the impact of the fly on the acoustic behaviour of the males. A field experiment shows that a species with a high syllable number were parasitized to a greater amount than species with one-syllable-songs at the same place. The singing and mating behaviour of parasitized compared to unparasitized males was analysed.

12-041

SEX APPEAL IN APHIDS

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Aphids usually have sex in autumn. In host-alternating species, winged males locate the wingless females using a complex of cues including a sex pheromone released from the hind legs of the females, as well as odour and visual cues associated with the plant on which the females reside. Our experiments with a field-based wind tunnel indicate that males can detect synthetic sex pheromone at a distance of 3-4 m and that they will fly upwind against wind speeds of 0.7 m/s. Recent field studies have also shown that males are attracted to sex pheromone released from water-traps, and that catches are increased when plant volatiles are co-released with the pheromone. In addition, the colour of pheromone-releasing traps affects efficiency; yellow, light green and orange catch more males than transparent, black, white, blue, dark green and red traps. The relative importance of the different cues appears to vary between aphids and will be illustrated with reference to three species.

12-042

CHEMICAL COMMUNICATION IN SCARAB BEETLES WITH RECIPROCAL BEHAVIORAL AGONIST-ANTAGONIST ACTIVITIES OF CHIRAL PHEROMONES

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Female *Anomala osakana* produce (S,Z)-5-(+)-(1-dec-enyl)oxacyclopentan-2-one, which is highly attractive to males; the response is completely inhibited by even 5% of its antipode. These two enantiomers have reverse roles in the *Popillia japonica* sex pheromone system. Chiral GC with an electroantennographic detector suggested that *A. osakana* and *P. japonica* each have R and S receptors. This novel mechanism of reciprocal behavioral agonist-antagonist activities of chiral pheromones appears to play a pivotal role in overcoming the species isolation problem inherent in the use of a single-constituent pheromone system in scarab beetles.

12-043

FEMALE PREFERENCES FOR MALE DRUMMING IN THE WOLF SPIDER *Hygrolycosa rubrofasciata* (Araneae, Lycosidae)

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In many arthropods, females choose their mating partners on the basis of the acoustic signals produced by the male. These signals are generally believed to provide the female with an indication of the quality of the signaller and thus they might serve as honest handicaps for females. *H. rubrofasciata* provides the perfect model system to study the costs and benefits associated to female choice in arthropods. Males produce a characteristic sound (audible to the human ear) by drumming their abdomen against dry leaves laying on the forest ground. The females respond by shaking their abdomens immediately after the male's drum, thus providing the observer with a very clear indication of which male's signal was chosen. We recorded male drumming under different laboratory conditions. Female preferences for different components of the male drumming as well as the variability in drumming components between different males were analysed. Preliminary experiments suggest that females prefer male signals of relatively higher energy content (higher drumming rate and volume) which are more costly to the male. Drumming quality has also been found to be positively correlated to male survival in the laboratory. It is therefore likely that male drumming provides the female with a reliable indicator of the male's quality.

12-045

SEX PHEROMONES AND REPRODUCTIVE ISOLATION IN LEPTOTHORACINE ANTS (HYMENOPTERA: FORMICIDAE)

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Sexual behaviour in palearctic ant species of the *Leptothorax acervorum* group involves female calling with emission of a secretion from the poison gland acting on males as an attractant and aphrodisiac. The present study included three independent species of the genus *Leptothorax* and four others which live in socially parasitic association with them. Reaction of males towards female pheromones was investigated using static and dynamic olfactometer designs. Specifically, interspecific efficacy of pheromones was studied to determine their role in reproductive isolation among syntopic species, in particular between social parasites and their hosts. Males of three inquiline parasites of the genus *Doronomyrmex* show no reaction to the poison gland secretion of their host *L. acervorum*. This is also true for *Leptothorax* males exposed to pheromones of *Doronomyrmex* species. In contrast, between *L. acervorum* and another parasite, the slave-maker *Harpagoxenus sublaevis*, some response of males to the other species' secretion is encountered. *H. sublaevis*, however, differs strongly from the other species treated here in the diurnal cycle of sexual activity. Pheromones are cross effective within *Doronomyrmex*, and also between the investigated *Leptothorax* species. Within *Leptothorax*, though, females do not recognize heterospecific males as potential mates upon contact. Thus, in the investigated species group, reproductive isolation between social parasites and their hosts is brought about by different pheromone blends and schedule of daily sexual activity, while pheromones do not play a role in separating sympatric species among *Doronomyrmex* inquilines or independent *Leptothorax*. The significance of these results is discussed as supporting the hypothesis of a sympatric divergence of social parasites from their hosts.

12-044

DRUMMING ACTIVITY AS AN HONEST SIGNAL OF MALE VIABILITY IN A WOLF SPIDER

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Conditional handicap models allow females to use male sexual traits as honest signals of male viability. The assumptions for honest signalling are that the traits are costly and that they reflect male phenotypic condition, which causes the optimal trait sizes being largest for the most viable males. However, the experimental evidence of the costs are scarce and phenotypic condition has not been manipulated at the same time. Male wolf spiders *Hygrolycosa rubrofasciata* court females audibly by drumming dry leaves with their abdomen. Females prefer to mate with males of high drumming rate. Males with high food level maintained their drumming rate at a high level, while males with intermediate and low food levels exhibited a reduction in drumming rates. Thus, phenotypic condition of the males affects their sexual signalling. We manipulated part of the males to increase their drumming activity by presenting females in proximity. These males suffered higher mortality and lost significantly more weight than other males confirming that drumming is costly to males. Additionally, males that drummed most actively within the increased treatment group survived better than less active males. Thus males of *H. rubrofasciata* vary in their ability to bear the costs of drumming, which gives an honest signal of male quality for females.

12-046

THE ENERGETIC COSTS OF CALLING IN *POECILIMON HOELZELI*, *POECILIMON VELUCHIANUS* AND *CONOCEPHALUS DISCOLOR* (ORTHOPTERA; TETTIGONIOIDEA) MEASURED WITH A HIGH-RESOLUTION RESPIROMETRY

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Acoustic signalling by males to attract conspecific females is widespread throughout the animal kingdom. Several studies have been made to measure the energy required for sound production. These energetic costs are of particular interest in male bushcrickets which produce large spermatophores, which add also to the male mating-costs. None of the methods used in the former studies are suitable for a direct measurement of the energy used for calling in a bushcricket with short calling songs. In our study we used a flow-trough respirometry system, with a fast responding CO₂-Sensor. By omitting water-absorbent we could increase the time-resolution of the system, so that every single action of the animal generated a single CO₂-Pulse. The costs of calling in *Poecilimon hoelzeli* which produces only single-syllable-chirps can now be calculated by integrating the CO₂ pulses caused by the song. In *Conocephalus discolor* a species with varying song length, we can compare costs of long and short songs. The measurements registered also interesting phenomena like discontinuous ventilation.

12-047

THE PHEROMONAL MECHANISM OF COMMUNICATION IN NEST SEEKING SWARMS OF HONEY BEES, *APIS MELLIFERA*
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Honey bees face dual problems of locating suitable nest cavities for swarms and then coordinating the flight of thousands of individuals to the nest site. Nasonov pheromone produced by worker bees is the key communication system in this process: it communicates the presence of a suitable nest cavity to other scouts searching for nest cavities; it leads the airborne swarm toward the cavity; and it marks the cavity entrance for arriving swarm bees. Although the pheromone is a blend of seven components, two components appear incidental to activity and the ratios of the remaining five components can fall within broad ranges without apparent loss of activity. Choice tests revealed that activity is proportional to pheromone concentration over a large range of concentrations. In tests using crossover designs, queen pheromone was shown to play no role in the nest seeking communication. Other odors, including nest cavity odors, play minor roles in the communication, except that insecticide and other repellent odors can block the pheromone effect. Experiments in progress to determine if Nasonov is truly a pheromone, or if it simply serves as an odor will be presented.

The hypothesized mechanism of pheromonal communication by Nasonov pheromone to nest seeking bees is: scouts that successfully locate a suitable nest cavity scent with pheromone to communicate the presence of the cavity to other scouts; the pheromonal signal acts by generating a large pheromone-containing airspace downwind of the nest cavity which attracts and orients searching scouts which enter the airspace; once the many pheromone alerted scouts of a swarm 'reach a consensus' concerning the best nest cavity, Nasonov pheromone released by flying scouts aids in communicating the flight path to the cavity; and, finally, at the nest entrance Nasonov pheromone alerts and attracts the landing bees to the cavity entrance.

12-049

RHYTHM OF SEX PHEROMONE OF THE ORIENTAL ARMYWORM *MYTHIMNA SEPARATA* WALKER
(LEPIDOPTERA: NOCTUIDAE), AND
NEUROHORMONE REGULATIONMengying Liu, Hong Dong, Xinwen Wang

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The calling periodicity and producing rhythm of sex pheromone of *Mythimna separata* were investigated at 25 °C, under LD 14:10 photoperiod. It was found that the calling activity of *M. separata* is very low during the first six hours of scotophase, and then increases sharply, particularly in the 4-5 day old females. The maximal sex pheromone titre of female *M. separata* was detected in the extracts of the pheromone glands in 4-5 day old females after 6 to 8 hours from the point of the initiation of scotophase.

Tests of discapitation and injection of the brain-SOG homogenate of the adult moths in their 5th scotophase showed that the pheromone titre remains a very low level (average 1ng) by discapitation, and is restored to the control value (average 16ng) after injection of brain-SOG homogenates. During photophase, the brain-SOG homogenate still has a pheromonotropic activity, which exists in the brain-SOG complex of both female and male *Mythimna separata* during both scotophase and photophase.

12-048

A STUDY ON THE PRECISE BLENDING OF PHEROMONE COMPONENTS OF *ANCYLIS SATIVA* LIU. (LEP.: TORTRICIDAE)Lian-chang Li, Gui-biao Han¹, Jie Li²

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Recently many reports demonstrated the importance of precise blending of sex pheromone components. The present study reports the results of determining the precise blending of the sex pheromone components of *Ancylis sativa* Liu and individual variation within the population of sex pheromone chemical communication system using analytical technique of single pheromone glands. The analytical results of 47 single pheromone glands showed the precise blending of 2 components, 60:40 (E₉₋₁₂:OAC:Z₉₋₁₂:OAC), C.V.=9.8%. The result showed that individual variation within the population is controlled within narrow limits.

12-050

THE FUNCTION OF FEMALE SEX PHEROMONES IN MATE SELECTION OF *OSMIA RUFA* (MEGACHILIDAE) MALESG. Dutzler, M. Ayasse

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The function of pheromonal recognition signals in the mating biology of halictine bees is well investigated. Females release species-specific pheromones which give males further information about kin and mating status. Although the biology of the solitary megachilide *Osmia rufa* has been intensively studied, almost no data exist about the chemical communication involved in the mating behaviour. The males search for receptive females at feeding areas and at the often aggregated nests. The females of this monoandric species usually mate immediately after emerging. In behavioural learning experiments, we studied the role of individual female odour signals in male mate selection.

Bioassays showed that newly emerged females were more attractive to the males in comparison to one or two day older already-mated females. Gas chromatographic analyses of cuticular washings of the body showed significant differences in the odour bouquet of unmated and mated females, possibly responsible for the different attractiveness of these female groups to the males. Further behavioural experiments of mate recognition showed that males were able to learn individual female odours during mating attempts and to use this information in further encounters: When a female was offered a second time to the same male, significantly less copulatory activity was observed than in the first test. In a second series of tests, a first female of a pair was replaced by either a sister or a nonsister of the same or of a foreign population. In this case the attractiveness of the second female was not diminished. The results of our behavioural experiments indicate that in *O. rufa* males do not discriminate among receptive females; however, they recognise mated females and avoid spending time in mating attempts with unreceptive females.

12-051

SELECTION GRADIENTS IMPOSED BY PHONOTACTIC PARASITOIDS ON COMPONENTS OF SONG IN THE FIELD CRICKET TELEOGRYLLUS OCEANICUS

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Acoustically-orienting parasitoid flies (Tachinidae: *Ormia ochracea*) impose a selection gradient on calling male field crickets (Gryllidae: *Teleogryllus oceanicus*) by using cricket mating advertisement calls to locate hosts. However, male crickets that remain silent risk losing potential mates. This creates conflicting selection pressures on song structure, which must continue to attract females while simultaneously reducing the attractiveness to parasitoids.

We recorded males in the field on three islands within the Hawaiian Archipelago, which differ in proportion of males parasitized. After recording, each male was collected and scored for the presence of parasitoids. Thirteen temporal variables were measured from a sonogram of each song. Non-linear discriminant function analysis identified several independent combinations of song variables that differed significantly between parasitized and unparasitized males. We discuss these selection gradients in the context of natural and sexual selection.

12-053

A SPERMATOPHORE STRUCTURED IN THE BURSA COPULATRIX OF SOME BUTTERFLY SPECIES AND ITS DEPLETION IN RELATION TO SUGAR CONTENT

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During copulation, the male of pierid butterflies fills first a white gel and then structures a spermatophore in the bursa copulatrix of the female. The sperm was ejaculated last, while sugars were observed throughout the copulation. The older male ejaculated a smaller spermatophore than the younger one. Variation in size and sugar content of spermatophores observed in field-captured monogamous females indicated that sugars were consumed immediately after copulation, and that the spermatophore was gradually eroded. Uninterrupted copula durations between virgin male and virgin female in the sulfur butterfly were successful copulations, lasting about 45 min in the laboratory. Most copulations were prolong in the field from those in the laboratory due to lone males harassing, though no male ejaculated a larger spermatophore. The lone males significantly increased the duration of a harassment of the pair that copulated with old males. No severe harassment elicited any particular behavior of the pairs. The female of the pair also ignored the lone males harassing. A few take-over of female by lone males harassing and a few females accepting re-mating with a lone male just after the first copulation were observed. Most of the females were copulated with old males. About 15% of experienced females in the field accepted further mating, requiring longer duration of courtship behavior by lone males than virgin ones. Females that accepted re-mating retained smaller ejaculates in the bursa copulatrix than those that refused re-mating. Severe harassments of pair with old males may be advantageous to lone males due to a small spermatophore ejaculated by the old males.

12-052

SEXUAL BEHAVIOUR AND MATING IN *BLATTELLA GERMANICA* LINN. (BLATTOIDEA ; BLATTELLIDAE)

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Exact sequencing of various steps in the courtship, sexual behaviour and mating, starting from the recognition of the potential mate, has been carried out in *Blattella germanica*, with the help of video recording. The males show some specific movements which can be described as courting the female. Some movements, representing sexual foreplay, are however, common to both the sexes. The female does not actually feed from the metanotum of the male, as is believed thus far. All movements of the female, during the process of mounting the male, are in the nature of tactile stimulation only. These movements commence from the last abdominal tergum and shift anteriorwards till they reach the metanotum, and involve tapping of the terga of the male by her maxillary palps. Attempt for interlocking of the genitalia is made immediately as the metanotum of the male comes under the maxillary palps of the female.

12-054

EVOLUTIONARY CHANGES IN NUPTIAL GIFT SIZE CORRELATE WITH EVOLUTIONARY CHANGES IN SPERM NUMBER IN BUSHCRICKETS (ORTHOPTERA: TETTIGONIIDAE).

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The spermatophores of bushcrickets consist of two parts: an ampulla which contains the sperm and a spermatophylax which the female eats following mating. Interestingly, there is a large degree of interspecific variation in the relative size of the spermatophylax. There are two different, though not mutually exclusive, hypotheses concerning the selective pressures important in the evolutionary enlargement of the spermatophylax in bushcrickets. The paternal investment hypothesis proposes that elaboration of spermatophylax size has proceeded through selection for male nutritional investment in their own offspring. The ejaculate-protection hypothesis, on the other hand, proposes that the evolutionary enlargement of the spermatophylax has proceeded through selection to ensure complete ejaculate transfer. The latter hypothesis predicts that evolutionary changes in spermatophylax size should be positively correlated with evolutionary changes in ampulla size (ie. ejaculate volume) and sperm number. Here I present the results of a comparative study designed to test this prediction. Measurements of spermatophylax mass, ampulla mass, sperm number and male body mass were taken for 43 species of European bushcrickets. The data were analysed using the independent comparisons method. This method corrects for similarity between taxa resulting from common ancestry and involves converting the data set into a series of statistically independent contrasts. As predicted by the ejaculate-protection hypothesis, a positive relationship was found, across taxa, between changes in spermatophylax mass and changes in both ampulla mass and sperm number, with male body mass controlled for.

12-055

OVIPOSITING STRATEGY OF *EUPEODES CONFRATER* (DIPTERA: SYRPHIDAE) FEEDING ON *PSEUDOREGMA BAMBUCICOLA* (HOMOPTERA: PEMPHIGIDAE) WITH STERILE SOLDIER CASTE

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Recent studies on aphidophagous syrphid flies demonstrated that females, in searching oviposition sites, assess qualitative and quantitative value of aphid colonies as suitable and sufficient food resource for their offspring. However, oviposition behavior of syrphid flies feeding especially on soldier-producing aphids is never intensively studied. My study on ovipositing strategy of *Eupeodes confrater*, which feeds on eusocial aphid *Pseudoregma bambucicola* with a special soldier caste, demonstrated that female flies selected large colonies for their food and laid eggs on various sites such as fine threads of spider webs and hairy objects in or close to aphid colonies. Since the aphid soldiers were able to attack syrphid eggs using their frontal horns, only the eggs laid on spider threads could escape. Female flies could change their oviposition sites in relation to soldier density. For instance, when the soldier ratio of aphid colonies was high, most of females did not lay eggs in or close to the aphid colonies, but on spider threads near the colonies. In contrast, when the soldier ratio declined (nearly 0%), females laid eggs directly on the colonies. These results possibly suggest that females of *E. confrater* select optimal sites for oviposition in response to the density of aphid soldiers.

12-056

THE INTERACTION BETWEEN THE EFFECTS OF TERRITORY AND SEX RATIO ON COPULATION DURATION IN THE WALNUT FLY *RHAGOLETIS JUGLANDIS*

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Ecological factors such as operational sex ratio (OSR) and territoriality are known to play important roles in mating dynamics. While most studies address the effect of each factor independently, the interaction between them is seldom considered. Using a combination of field and laboratory assays, we examined the role of both factors in the mating dynamics of the tephritid fly *Rhagoletis juglandis*. This species is characterized by a resource defense mating system in which males defend walnuts and mate with females that arrive on the fruit to lay eggs.

In the field, copulation duration in *R. juglandis* was distributed bimodally, most copulations being either short (≤ 200 sec) or long (> 600 sec). In both field and laboratory, OSR had a strong effect on copulation duration: at male biased sex ratios, copulations tended to be long; at even or female-biased ratios, copulations tended to be short. Individual flies were able to shift copulation duration in response to changes in sex ratio occurring over a scale of a single day.

In a final laboratory assay, the effect of OSR on copulation duration was found to depend on the presence of fruit. In the presence of surrogate fruit, the usual effect of sex ratio on copulation duration was expressed; in the absence of such fruit, however, the effect disappeared. In the same assay, the effect of fruit presence on copulation duration depended on OSR. At a female-biased ratio, the presence of a surrogate fruit tended to result in relatively more short copulations. At a male-biased sex ratio, however, the presence of fruit had no effect on copulation duration.

12-057

The Effect of the Oviposition Behaviour on the Fitness of *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae)

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For the observation of the effect of the oviposition behaviour of bean weevil (*Callosobruchus maculatus*) on its fitness on azuki beans and mung beans, three strains from Taiwan, 4C6-4, 13M23 and 10CG-8, were used. These geographically different strains showed significant differences in the longevity, the fecundity, the adaptation ability to hosts and the degree of larval competition. Because of the non-feeding culture in adult stage and the lack of the ability of self-dispersal in larval stage, these differences will directly influence the fitness, which was defined as the number of offsprings. All the above reproductive characters were described quantitatively and used to compare the fitness of the three strains among two hosts. A random egg distribution simulated by computer according to the observed fecundity was also compared with the observed egg distributions to enumerate the benefit of the oviposition behaviour. It was found that the female bean weevils of all three strains tended to lay their eggs in a nearly uniform distribution and their fitness was increased by this oviposition behaviour, but the connection between larval competition and egg dispersion was lost. It suggests that although the behaviour of uniform egg dispersion should be a result of selection, the cost of barring this behaviour could be relative low when compared with its benefit.

12-058

NO HOST-QUALITY PREFERENCE BY THE HYPERPARASITOID, *EURYTOMA* SP. (HYMENOPTERA: EURYTOMIDAE), FOR THE COCOONS OF THE PARASITOID, *COTESIA GLOMERATA* (HYMENOPTERA: BRACONIDAE)

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Eurytoma sp. is a solitary hyperparasitoid which attacks *Cotesia glomerata* cocoons of various ages. Under laboratory conditions of 20°C and 16L8D photoperiod, the number of eggs laid per day in old cocoons was nearly the same as that laid in young ones. However, females laid more male-eggs in old cocoons than in young ones. The developmental period of *Eurytoma* sp. was longer and the mortality was higher when laid in old cocoons than those laid in young ones. Apparently, the old cocoons were less suitable for *Eurytoma* sp. than young ones.

However, *Eurytoma* sp. females did not show any preference for good cocoons when offered young and old host cocoons simultaneously.

Investigations of *C. glomerata* cocoons in the field showed that the hosts of many clusters developed relatively equally. Further, the longevity of the female *Eurytoma* was as long as 2.5 months or more at 20°C. The number of eggs laid in a day by young females was nearly 10. Even old females could lay several eggs in a day. The ability of egg production was considered high for both young and old females. Therefore, under natural conditions, it might be advantageous for females to lay eggs immediately after encountering the host cocoons.

12-059

REPRODUCTIVE BEHAVIOR OF THE PAPAYA FRUIT FLY *TOXOTRYPANA CURVICAUDA* (DIPTERA: TEPHRITIDAE) IN THE CENTRAL MEXICO.
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Full understanding of insect behavior and its relevance to the Integrated Pest Management process will lead eventually to application of more effective and safe pest management practices.

The papaya fruit fly, *Toxotrypana curvicauda* is the main pest of papaya crop in central part of Mexico.

Studies were conducted at field conditions in order to know dispersal behavior and in laboratory to know reproductive behavior.

Ethograms were made considering the relative frequency of 21 patterns. Results showed a low dispersion of adult in the crop. The flies were found mainly at the border of the papaya crop. Dispersion did occur at 17:00 hrs. and flies arrived to the papaya grove at 11:00 hrs next day. At laboratory, patterns of behavior differs significantly between males and females. Patterns were found in higher numbers as the flies became older. In males, patterns with high frequency were setting, grooming and calling while in females, they were setting, flying, mouthparts moving.

12-061

WHY A MALE CRICKET COURTS ANOTHER MALE ?

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Adult males of *Gryllus bimaculatus* court their partner independently of their sex. The courtship being only if the partner is immobile, whether female, subordinate male or a dummy (male or female anaesthetized by carbon monoxide). The main stimulus which provokes courtship therefore seems to be the behaviour (immobility) of the partner.

The frequency of courtship between two males ("homosexuality") decreases as the available surface increases. We conclude that the greater the possibility subordinates have to escape, the less likely they are to become immobile when confronted by a dominant partner.

In the context of Evolutionary Game Theory, immobility can be considered as a tactic, adopted by subordinates, to inhibit aggressivity of dominant individuals when escape is not possible or too costly.

12-060

THE ROLE OF NUTRITION AND JUVENILE HORMONE IN REGULATING MATING BEHAVIOR IN BOTH SEXES OF *PHORMIA REGINA* (MEIGEN)(DIPTERA: CALLIPHORIDAE)
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Mating behavior of both sexes of *Phormia regina* is dependent on nutrition, which in turn influences the corpora allata to produce juvenile hormone (JH). Sugar-fed flies do not exhibit mating behavior while protein-fed flies mate. Approximately 10 mg of liver was required for 80% of the males to inseminate females whereas 20 mg of liver was required for 78% of the females to become inseminated. For females, less protein is required to activate sexual receptivity (i.e., 10 mg) than to develop mature eggs (i.e., >15 mg). Allatectomy greatly suppressed mating behavior of both sexes. Topical application of 10 ug of methoprene (a JH hormone analogue) at 12 h after the onset of liver feeding significantly increased sexual activity of both allatectomized males and females.

12-062

REPRODUCTIVE STRATEGY AND VARIABLE MATE GUARDING BEHAVIOUR OF GYPSY MOTH, *LYMANTRIA DISPAR JAPONICA* L. (LEPIDOPTERA, LYMANTRIIDAE).

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Male and female reproductive strategies were investigated in a Japanese population of the gypsy moth, *Lymantria dispar japonica* L. in the laboratory and field.

During the days, mated females stayed motionless at the copulation sites. At dusk, the females began to walk and/or fly actively in search of oviposition sites. Oviposition began at night and continued for several days at the same site. Females ceased oviposition every morning after covering the eggs with a thin layer of scales from their abdomens. In the field, females showed multiple mating before oviposition.

Gypsy moth in Japan, show two patterns of copulation; short duration and long duration. Short duration copulation was sufficient to fertilize almost all the eggs of a female. Long duration copulation continued until a female began pre-ovipositional behavior and occurred when other males were attracted to the copulating pair. Extended copulation, therefore, appears to be a mate guarding strategy by males. Males change their strategies from mate searching to mate guarding according to the disturbances by other males. I also compared the reproductive strategies of the gypsy moth with those of other temperate and tropic diurnal moths.

12-063

MALE MATING BEHAVIOR AND A CONTACT SEX PHEROMONE OF THE YELLOW-SPOTTED LONGICORN BEETLE, *PSACOTHEA HILARIS* (PASCOE) (COLEOPTERA: CERAMBYCIDAE).

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A male of the yellow-spotted longicorn beetle, *P. hilaris* recognized a female when he directly contacted her with his antennae, tarsi and/or palpi. A contact pheromone revealed to be essential in releasing male mating behavior of this beetle. The female sex pheromone consisted of at least two factors with different functions, the one caused dash behavior toward female, and other did holding, mounting and abdominal bending behavior. A major component of the pheromone was isolated from cuticular hydrocarbon fraction of female elytra extract. It was identified as (Z)-21-methyl-8-pentatriacontene, which accounted for ca.60% of the hydrocarbon components. The synthetic compound released the typical mating behavior including holding, mounting, and abdominal bending in males when treated on a gelatin capsule as an artificial female dummy. The activity was the component, however, considerably lower than the extract of female elytra.

12-065

MALE REPRODUCTIVE BEHAVIOUR IN TWO SPECIES OF *SYSTROPHA* BEES (HALICTIDAE)

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Populations of the two solitary bees *Systropha planidens* and *S. curvicornis* are very rare in Central Europe. Females of both species can often be found in mixed nest aggregations. Females of the oligolectic species tend to forage mainly in the morning on flowers of *Convolvulus arvensis*. No information exists about the mating biology. We investigated the mating biology of both species by behavioural observations and field experiments.

Flowers are very often encounter sites in oligolectic bees. In fact, we found patrolling males of both species moving constantly along fixed routes between flowers of *Convolvulus arvensis*. The flight routes of single males overlap within and between species. Territory owners strike intruding conspecific males as well as *S. curvicornis* males with their last sturdy tergites. In behavioural experiments, dead males and females of both species, previously killed by freezing, were offered to territory owners by placing them in *Convolvulus* flowers. The observed male behaviour indicates that the odour bouquets on the cuticular surface are species-specific and encode information about sex: *S. planidens* males tried to mate with conspecific unmated and already mated females, and showed more aggressive behaviour towards conspecific males in comparison to *S. curvicornis* males. The hypothesis that the odour bouquets on the cuticular surface encode sex- and species-specific information is presently being investigated by chemical analyses.

12-064

MATING STRATEGIES AND MATE SELECTION BEHAVIOUR OF *DYSDERCUS KOENIGII* F.

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The female of *Dysdercus koenigii* exhibits multiple mating behaviour. After attaining sexual maturity the bugs were observed to mate 5-7 times. The first mating was of the longest duration. The copula lasted up to 72 hr. The females although were mating - receptive throughout their life, tended to be refractory for mating after copulation. Mating refractory behaviour of a female was shown to depend on the duration it remain in copula. A female, for example was allowed to mate for 24 hr after which it still remained receptive to mating. However, 48 hr mating evoked a mating refusal behaviour which was more pronounced in the female that mated for 72 hr. Renewal of mating receptivity occurred in the female after oviposition. Regarding the mate-selection behaviour of the female, it was observed that it mated with any male encountered first. In a choice test a male which had earlier mated for shortest duration would prefer to mate again. The male on the other hand preferred to mate with either a virgin female or 24 hr mated female. Significance of male substance in suppression of mating receptivity in the female and possible evolutionary advantage of mate-selection behaviour of male *Dysdercus koenigii* is discussed.

12-066

STUDIES INVOLVING THE MECHANISMS OF "SPERM PRECEDENCE" IN *TRIALEURODES VAPORARIORUM* (HOMOPTERA: ALEYRODIDAE)

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T.vaporariorum males have been radiosterilized by means of "gamma rays" to a dose of 50 Gy.

At this dose males preserve a partial spermatozoid competitiveness and sperm transfer capacity. However spermatozoa are unable to fertilise eggs and produce viable embryos.

We have analysed the egg mortality and the sex-ratio in progeny born in the following mating types:

A: virgin females x normal males

B: virgin females x sterile males

C: virgin females mated firstly with normale males and then with sterile males.

We found a significant higher mortality in B and C (about 35%) compared to A (0.2%). We considered the major egg mortality found in B and C to be a measure of the fertilization capacity of spermatozoa produced by sterile males.

Moreover, we found both the sexes in progeny produced in mating A, while an exclusively male off-spring was born in mating B.

The sexual analysis of progeny produced in mating type C, has showed, in the first days of adult emergence, a completely male off-spring. Subsequently rare females have appeared. According to these results we may suppose that the spermatozoa of the most recent matings were favored in the fertilization process.

12-067

THE NESTING BEHAVIOUR OF CENTRIS (HETEROCENTRIS) ANALIS (FABRICIUS, 1804) (HYMENOPTERA, ANTHOPHORIDAE).

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The nesting behaviour of Centris analis was studied on the University Campus of Ribeirão Preto, SP, Brazil. The bees nested in trap-nests made with black cardboard having 5.8 cm in length and 0.6 cm of internal diameter introduced into holes made in wood plates which were placed on the shelves of two shelters built at the study site. After a suitable trap has been found, the female collects oil to line internally the trap. Subsequently she begins to collect plant material to construct the bottom wall of the nest and the beginning of the first cell partition in the form of a small ring that encircle all the circumference of the nesting tube. The plant material consists essentially of pollen, anther fragments, filament, shaving of pollinic sacs and fibres. Four to 11 pollen-collecting trips are made to provision one cell. Cells that produced females were provided with more trips of pollen than those that produced males. After all the pollen has been deposited, the female collects additional plant material and adds it to the cell ring. After that the female performs from 2 to 8 nectar-collecting trips to complete the provision of the cell. No difference was observed between the number of nectar-collecting trips for female and male cells. After depositing nectar on the provisions, the female oviposits and immediately seals the cell by constructing the cell partition from material previously deposited on the cell ring. The number of brood cells in each nest ranged from 1 to 4, usually followed by an empty vestibular cell. The nest plug is constructed of the same material as partitions and it is lined externally with oil. The time needed to complete a cell ranged from 1 to 5 days, but mostly within one day. The maximum number of nests and cells built by a female were 3 and 9, respectively. The largest period in which a female remained nesting in the area was 30 days.

12-069

ASSESSMENT OF APHID COLONIES BY THE HOVERFLY
(EPISYRPHUS BALTEATUS (DE GEER))(DIPTERA: SYRPHIDAE)

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In the syrphid hoverfly Episyrphus balteatus (de Geer), females in search of oviposition sites are assessing the qualitative and quantitative value of aphid colonies for securing the successful development of their offspring. They select small, young and "promising" aphid colonies consisting of nymphs and/or fundatrices as their oviposition sites, and neglect large, older ones including winged adults. Such "buy-futures" tactics will benefit the offspring in finding suitable and sufficient food.

12-068

THE NESTING ACTIVITIES OF CENTRIS (HEMISIELLA) VITTATA LEPELETIER (HYMENOPTERA, ANTHOPHORIDAE).

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Observations on the nesting activities of Centris vittata were conducted at Santa Carlota Farm, Município of Cajuru, SP, Brazil. The bees nested in bamboo canes which were placed along shelves in a shelter built at the study site. The diameters of canes utilized (n=67) varied from 1.0 to 1.9cm and their lengths from 8.9 to 22.2cm. Once a cane is selected, the female begins to forage for nest construction material that consists of a mixture of soil+oil. Thirty-seven to 53 material-collecting trips are required to construct one cell and the beginning of the cell cap that encircle part of the cell opening. Material collecting trips lasted 35-1754 sec. (n=200). After the cell is built, the female performs a variable number (9-14) of oil-collecting trips to line internally the cell. Oil-collecting trips lasted 76-3096 sec. (n=88) Twelve to 22 pollen-nectar collecting trips, which lasted 75-3427 sec. (n=144), are made to provision one cell. Total time spent in cells to deposit nectar and pollen loads ranged from 33 to 902 sec. (n=140). After cell provisioning is completed, the female collects additional oil (1 to 5 oil-collecting trips) and deposits it on the cell cap partially constructed. After that female lays the egg and immediately seals the cell from material previously there deposited. Following the same sequence, a serie of cell (from 1 to 6 cells) are constructed in a cane. Female progeny are normally produced in the inner cells and the males in the outer cells of each nest. C. vittata nested during most of the hot and wet season (September-April).

12-070

GLUING EGGS ON ANOTHER'S BACK IN
PHYLLOMORPHA LACINIATA (HETEROPTERA, COREIDAE): - EGG DUMPING OR PATERNAL CARE?
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Females of Phyllomorpha laciniata Vill. lay eggs on the backs of conspecifics, females and males. In nature males carry twice as many eggs as females. Eggs do not survive in nature undefended but survive rather well on an individual's back. Experimentally, I tested if females lay eggs on the backs of their mating partner after mating as expected if egg carrying is paternal care. If sex ratio was even, females laid eggs randomly with respect to paternity. However, when sex ratio was male biased most eggs were laid on mating partner's back. Some individuals rubbed off eggs from their backs by brushing their backs against different objects. Individuals that had five or more eggs rubbed off eggs more often than individuals carrying only a few eggs. A male's voluntary egg carrying (when several males competed over access to a female) is more likely to be explained as post zygotic mate guarding or mating investment rather than paternal care because some males rubbed off their own eggs. The field data and laboratory experiments convince that P. laciniata is not a species with exclusive paternal care in contrast to what is assumed in the literature. I conclude that females have adopted a "cuckoo" reproductive strategy by gluing eggs on another individual's back and "forcing" others to carry them. I will discuss on the possible evolutionary explanation on why females accept other female's eggs and why males accept eggs they have not fertilized.

12-071

NESTING AND NESTS' PROVISIONING IN *OXYBELUS ARGENTATUS* (HYMENOPTERA: SPHECIDAE) IN ALTA VALTELLINA (NORTHERN ITALY)

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During three summers from 1993 to 1995 about 50 nests of the solitary wasp *Oxybelus argentatus* have been observed at Le Prese (Alta Valtellina, Sondrio province, Lombardia).

All the females and their nests have been marked. Each nest has been observed since the beginning of the digging to the final sealing. Daily wasp behaviour was recorded since early morning, before the first wasp left, to late afternoon when all the activities stopped.

The study has been focused on the duration of the following actions: nest digging, nest provisioning, and nest closure. Inferences about prey number in each cell and cell number in each nest have been drawn.

Collected preys have been listed.

12-072

BUILDING ACTIVITY IN *POLISTES* WASP : LOCAL STIMULUS, SHAPE OF THE NEST AND CONTEXTUAL EFFECTS

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The building activities, owing to the long-lasting marks they leave, are especially suited to the study of how an animal makes itself acquainted with its environment in order to determine its behaviour. The studies available clearly show the influence of stimuli received locally, but, frequently do not allow to distinguish their direct releasing influence from the effect of the context in which the reception occurs.

Our experimentation aims at testing the effect of such contextual influences. In a succession of experiments, we offered to digynous *Polistes* colonies several devices with structures that differ from natural nests concerning either local stimulus or general outline : inverted nests, nests associated back to back... In these situations were studied the effects of several parameters : flatness of the comb, presence of a colonial chemical mark, orientation to gravity...

These effects were evaluated by the subsequent building behaviour and the shape of the newly built structures. These results are interpreted in terms of local stimulations and contextual influences.

12-073

NEST RECOGNITION AND ASSOCIATED BEHAVIOUR OF TWO SPECIES OF GRYLLACRID CRICKETS (ORTHOPTERA : GRYLLACRIDIDAE).

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Gryllacridids are a group of primitive, nocturnal crickets that are unusual in their ability to form 'nests'. These nests, constructed from silk secreted from an individual's mouthparts, represent a costly investment by the animal. Individuals of both sexes would therefore be expected to be able to recognise their own nests, and defend these nests from conspecifics.

Observations on *Ametrus* and *Hadrogryllacris* species reared in the laboratory show that nest construction and associated behaviour commonly occurs within 4 days of hatching. Thus, nests appear to be an important resource for juvenile crickets of these species.

At the onset of daylight, juveniles return to their own nests more than 55% of the time. Those that fail to return spend the day outside of any nest; occupation of a conspecific's nest is extremely uncommon. Research has shown that pheromones play an important part in recognition of nests. However, spatial cues (indicative of memory) and/or behavioural interactions may also be important factors in nest recognition.

12-074

NEW INSIGHTS TO FOOD AND OVIPOSITION PREFERENCE OF THE BUSHCRICKET *PHANEROPTERA FALCATA* (PODA) (ENSIFERA: PHANEROPTERIDAE)

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So far, in Central Europe *Ph. falcata* is generally characterized as connected with bushes on semiarid grassland. All over the literature this connection is explained as a result of oviposition inside of leaves of several shrub species. In the present study, the actual relation of *Ph. falcata* to habitat was investigated with regard to oviposition and food preference. Some laboratory experiments and field studies were conducted between 1992 and 1995.

In first laboratory experiments, some species of shrubs were offered to the bushcrickets in several combinations. A distinct oviposition preference to some plant species could be proved. Also the insects fed the leaves of offered shrubs in regular patterns and preferred some plants.

In contrast, the field study showed a clear food preference to blooms of herbaceous plants. Surprisingly, in the natural habitat the females chose almost only leaves of grasses (Gramineae) for the oviposition. Laboratory experiments clearly confirmed this behaviour. The bushcrickets laid their eggs not only inside of grass species of the semiarid sites (*Bromus erectus*, *Brachypodium pinnatum*), but in grasses of fresh meadows too (*Dactylis glomerata*).

In Further laboratory experiments the food preference observed in the field study could be proved as well. Firstly, *Ph. falcata* preferred blooms of mostly herbaceous plants. Secondly, the species chose faded blooms, fruits and seeds. Not until these food sources had been removed or got very dry, the bushcrickets fed leaves of shrubs and herbaceous plants in larger quantities.

Therefore, with regard to oviposition and food, a relation of *Ph. falcata* to scrubby vegetation could not be proved. The obvious occurrence of the species on such habitats may be discussed as a covered influence of the habitat management. The eggs of the bushcrickets laid inside the leaves of grasses would be removed by any mowing in autumn or by intensive grazing. Thus, a less management allows the survival of *Ph. falcata*, but is combined with an increase of bushes.

12-075

SPIDER MITE INFLUENCE BEHAVIOR OF ONION THRIPS ON CUCUMBER CROP UNDER GNEENHOUSE CONDITONES.

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Spider mite, *Tetranychus urticae* K. and onion thrips, *Thrips tabaci* L. often invade cucumber plant in association with each ther providing that both species are present in a greenhouse. This phenomenon might be observed in some cases even in the situations approaching to ecological vacuum. The behavior of *Th.tabaci* has been studied in free choice experiments during the investigation; spider mite invaded cucumber plants were compared to intact ones.

It has been shown that thrips response to the presense of spider mites on cucumber leaves depends on stage of insect development: mainly negative response was notified for adults while thrips larvae responded positively. It has been known that food specializations of adults and larvae of onion thrips differ to some extent. In particular, the ability of thrips to play the role of mite predator is characteristic of larvae only. The preferences of thrips larvae in relation to the host plant are rather similar to that of spider mite. Larvae of the insect prefer to feed within mite locus if the latter is available on the cucumber leaf. In the case if locus square is over than 3-5 cm² thrips larvae prefer to feed in its border areas. As a rule the share of larvae feeding within mite locusus is higher on cucumber genotype being resistant to the both pest species as compared to that on sisceptible plants. It is probable that the same immunological barriers might be involved in interactions of cucumber plants with the both pest species.

12-077

VISUAL CONTROL IN THE GIANT HONEYBEE *APIS DORSATA*: THE OCELLAR SYSTEM CONTRIBUTES TO FORAGING RECRUITMENT
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Honeybees start to forage nectar or pollen under conditions that are given mainly by colony factors, weather and season. However, the decision to dare a foraging flight is not made by the colony but by the individual itself. The individual factors releasing foraging activity deal in particular with the interplay of exploiting a food source in accordance with its profitability. A source of food may become unprofitable if e.g. the flow of nectar is diminished or otherwise, if visual orientation becomes intricate for the forager bee to find the path to the food source or back to the hive. It is an open question whether besides the compound eyes also the ocellar system is engaged in managing such tasks of visual orientation. We studied the possible roles of the ocellar system in *Apis dorsata* in visual orientation by stepwise modifying the stage of difficulty for securing food. We investigated the behavior of two differently treated groups of trained fully sighted bees, of one group of trained ocelli-occluded bees and one group of newcomers, when flying through apertures - variable in size - to come to a feeder or back to the hive. The results of two experiments (1994,1995) comprise more than 30.000 observations and show that the bees will stop revisiting the feeder if visual orientation gets difficult for them to a certain extent. Hereby, the ocelli-occluded bees stay away from the feeder at lower stages of difficulty (at wider openings) than fully sighted bees do. This is plausible as occlusion of the ocelli makes the bees clumsy regarding visual orientation, which can be observed e.g. in their lower capability for adjustment of the flight course during foraging. On the other hand, ocelli-occluded bees hold the memory of a food source, as they revisit the feeder on consecutive days obviously to check its profitability. This is the first evidence for a role of the ocelli in controlling the recruitment of forager bees. Moreover, the data disclose that the behavioral differences of ocelli-occluded bees are caused specifically by the lack of ocellar functions and not just by their discomfort through the dye on their heads.

12-076

ASSOCIATIVE LEARNING BY ANTHOCORID PREDATORS PLAYS A ROLE IN PREY ODOUR RECOGNITION.

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Anthocorid predators (*Anthocoris nemoralis* Fabricius, *A. nemorum* (L.) and three species of *Orius*) respond to odours emitted by pear trees attacked by pear psyllids (*Cacopsylla pyricola* (L.)). Predators seem to be rather variable with respect to their response to the compounds present in the odour blend emitted by trees. If adult anthocorids are collected in pear trees, the majority (75-96%) is attracted to methyl-salicylate -one of the compounds present in the odour blend- over clean air in a Y-tube olfactometer. If they are collected in the hedgcrows surrounding the orchard, fewer individuals (60-75%) seem to respond positively to the compound. However, the response of captive *A. nemoralis* adults reared on a diet of lepidopteran eggs hardly exceeds 50%. These findings suggest that expericnce with odour cues may play a key role in prey recognition and prey finding by the bugs. In a series of experimnts it is demonstrated that associative learning is not limited to hymenopteran insects such as honey bees and parasitoids, but can also be found in anthocorid predators.

12-078

ORIENTATION RESPONSES OF THE PREDARORY BUG *PERILLUS BIOCULATUS* TO VOLATILE BLENDS FROM THE POTATO-COLORADO POTATO BEETLE COMPLEX

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The predatory bug *Perillus bioculatus* Fabricius (Pentatomidae: Heteroptera) is a specialized natural enemy of chrysomelid beetles, such as the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say (Chrysomelidae: Coleoptera). We studied the locomotory behaviour of *P. bioculatus* in response to different stimuli using an automated locomotion compensator. Males or females were exposed to a stream of clean air (speed 0.25 m/s), to air carrying the odours of intact or mechanically damaged potato plants and to air passed over plants infested with feeding or non-feeding CPB larvae. Air passed over intact potato plants evoked locomotory responses similar to clean air. Air passed over the potato-CPB complex elicited distinct orientation responses from *P. bioculatus*, characterized by significantly higher values of upwind fixation (a measure for upwind orientation i.e. upwind length divided by track length) than those displayed in response to volatile blends from intact potato plants or clean air. *P. bioculatus* responded to odours emitted by mechanically damaged plants in a way similar to the CPB-potato complex, but this response disappeared within 1 hour after damage was inflicted. In contrast, feeding damage produced oriented locomotion for several hours after removal of CPB. Gas chromatographic analyses of headspace volatiles emitted by intact potato plants and plants damaged either mechanically or by feeding CPB, showed that several volatiles are emitted only after feeding by CPB and that the release of these compounds is sustained for several hours after removal of CPB larvae. No consistent differences in locomotory behaviour were found between the sexes.

12-079

HOW VISUAL SHAPE SURROUNDING THE NEST HELP FOR NEST LOCALIZATION BY THE SOLITARY BEE, *MEGACHILE ROTUNDATA* (F.) (HYMENOPTERA: MEGACHILIDAE)

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Female of leaf-cutter bee, *Megachile rotundata*, has to localize its nest after each foraging trip, carrying a piece of alfalfa leave, nectar or pollen. Guiding is about exclusively visual. We have experimentally measured, in natural conditions, in which respect visual cues closely surrounding the nest participated in guiding the final approach before landing on the familiar nest hole.

The experimental device consisted of styrofoam nesting blocks (10X10cm side area) with 49 spaced holes filled with a soda straw. Each block was evenly flushed at the centre of a 40x40cm metal platform hanging vertically. Nine of these devices were spaced in an horizontal row. Each nest block was surrounded by 4 geometrical shapes, fixed with magnets, then movable and removable. The experiments were conducted by disturbing or removing the 1-4 shapes.

The results showed that nesting females used a limited extent the shapes pattern surrounding their nests, and each individual shape as well. Nest localization was delayed but never prevented. Removing from 1 to 4 shapes increased the delay, but the effects of the positions of the removed shapes appeared unclear. Pivoting the whole pattern does not make any difference. The results will be discussed within the frame of the snapshot theory.

12-081

ORIENTATION BEHAVIOUR IN *CATAGLYPHIS CURSOR* (HYMENOPTERA, FORMICIDAE) AT THE INDIVIDUAL LEVEL

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Many studies of orientation in insects have emphasized their powerful abilities for finding their way home. In the ant *Cataglyphis cursor* for example, Pastergue-Ruiz et al. (1995) have shown that foragers are able to home in a strictly controlled laboratory environment using only visual cues. Whereas in most of these latter studies orientation has been measured as the average performance of a group of individuals, the aim of our experiments was to study specifically the individual performance of the workers.

Our main purpose was to test the well-known "snapshot model", which postulates that insects use a snapshot-like representation as a template to locate particular places of their environment (Collett, 1993). Homing trips of individually marked ants were recorded and studied using computerized methods allowing a fine statistical track analysis. A particular algorithm was used to detect the "breaking points" of the tracks, i.e. the points where a switch in the path characteristics occurred from a search to a more direct trajectory. We hypothesized that from these points on ants had identified and were able to use the cues they had memorized to reach their goal. By mapping the location of these points for several successive trips of the same ant we were able to delimit the "catchment area" characteristic of each individual, i.e. the area where adjustment between the memorized template and the retinal image becomes possible. This method allowed us to measure the flexibility of the localization system, and therefore to infer the cognitive processes underlying orientation tasks in ants.

This study shows in addition that within the same species of ants several different navigational strategies can be used, some of them based on kinesthetic, others on visual cues.

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12-080

EFFICIENCY OF SOME INDUSTRIAL DEVICES FOR NEST LOCALIZATION BY THE LEAF-CUTTER BEE, *MEGACHILE ROTUNDATA* (F.) (HYMENOPTERA: MEGACHILIDAE).

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Megachile rotundata is broadly used for alfalfa pollination by the seeds producers in North America. Large quantities of bees are released in alfalfa crops and the females are offered to nest in various devices differently efficient in allowing a female to find its own nest. The more efficient is the localization the shortest the rate of the cycle and the pollination process.

Three types of devices were evaluated in this respect. First, a shelter (2mx1.5mx1.5m), one of its long side opened and a trailer (5mx4mx3m) opened to its back wall. Both devices were settled in the crops and provided with several styrofoam boards for nesting. A third device consisted of 5 roll-a-boards (40cm diameter), clustered on a pillar in a garden, and made of rolled undulated cardboard.

These devices appeared very poor for nest localization. Because of the high density of nests holes and the large number of insects in the same area, the bees were wasting time and energy searching for their own nest and fighting with conspecifics. The homing efficiency could be drastically improved by additional visual cues and by reducing board size and density of nest holes. Improving the device should allow a decreased of the number of bees required for pollinating an alfalfa crop.

12-082

DO INSECTS HAVE EXPECTANCIES ?

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Workers ants of the species *Cataglyphis cursor* forage individually using terrestrial visual cues. Former laboratory experiments indicated that *C. cursor* can encode and store different visual landmarks, as geometrical forms, to locate the direction of their nest (Pastergue-Ruiz et al, 1995). In the present work we constrained the ants to follow a route to find their way back home. Four sequentially separated rooms (A, B, C, D), connected with plastic tubes, linked a food site to the nest. In each room, ants were confronted with a spatial binary choice corresponding to the entry of one of two plastic tubes, one leading to a dead-end ("wrong" choice) and the other one to the following room ("correct" choice), and this up to the nest entry. Each of the two issues in each room was identified by a specific visual landmark, i.e. a cross (indicating the correct entry) versus a circle (indicating the wrong entry) or a triangle versus a square, and so on. The ants rapidly learned the correct sequence of visual cues and homed quickly. When the four rooms were permuted (C, D, B, A), workers homed correctly but were disturbed with many back and forths between the box D and the box B (one of the two landmarks in the box D indicating the nest in the learning condition). Thus ants bear more care on the last cue of the sequence which is spatially and temporally linked with the nest entry. In other words, ants behavior seems to indicate a kind of expectancy of an event: i.e. the entry in their nest at a given time and place.

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12-083

HOST PREFERENCE IS A PHYSIOLOGICAL AND NOT A BEHAVIORAL TRAIT.
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Host preference, which is often measured as the number of eggs females lay on different hosts, is considered a behavioral trait. Until now the only physiological factor tested on host preference was egg load. We studied the physiological effect of the host on the rate of oocyte maturation in the potato tuberworm (PTW), *Phthorimaea operculella*. When its host is unavailable, PTW readily lays eggs on low quality hosts or even on non-hosts. Among its hosts, potato is the most preferred and tomatoes the least. Starved and 10% sucrose solution fed females were allowed to oviposit on potato tubers or tomato foliage. Females were dissected the following morning and the number of mature oocytes counted. Across all hosts, 54% of the starved females compared to 5% of the fed females had no mature oocytes in the ovaries. The number of mature oocytes in fed females on potatoes was higher than on tomatoes. Fed females laid significantly more eggs on potatoes than on tomatoes. Starved females laid similar numbers of eggs and had similar numbers of oocytes on both hosts. So both female feeding condition and the type of host affected the number of available eggs. We therefore conclude that the PTW lays all its available eggs during the night, regardless of which host it is on. Thus host preference in the PTW reflects physiological condition and is not a behavioral trait. We hypothesize that host preference measured as the number of eggs laid might be physiological in other synovigenic insects that readily lay their eggs on any oviposition substrate.

12-085

GENERALIZATION, DISCRIMINATION AND PREFERENCE AMONG FIGURES IN *BOMBUS TERRESTRIS* L. AND *APIS MELLIFERA* L. (HYMENOPTERA: APIDAE).
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The capacity of honeybees and bumblebees to generalize some features of shapes was investigated. The bees were trained to suck up the reward (50% sugar solution) through a capillary hole (Ø 1.5 mm). All the figures presented in the following experiments were fotocopied on a white paper and had a small hole in the centre which allowed the bees to reach the capillary. After a two-figure (test 1) and a group (test 2) training to discriminate between rewarded stars and unrewarded circles, the star was substituted with a five segment figure (test 3), then reduced to three (test 4), two (test 5), one (test 6), then five irregularly grouped ones (test 7), all rewarding figures. Two unrewarded figures were contemporarily presented in each test, both with a continuous perimeter. In the behaviour of bumblebees a generalization process occurred in the choice and explains the high percentages of landings on the segment-made figures in the beginning of tests 3-7, whose trends do not resemble a learning process. Honeybees showed much more difficulty in extracting the segment as the common element of the generalization sequence and they could not follow the complete breaking down of the segment-made figure. Showing the honeybees the one-segment figure caused them to stop their visits to the experimental plate. In order to explain the different behaviour of the two bees, discrimination and preference tests were performed. The 6 pairs of filled-up or empty figures the insects were offered in the generalization experiment were directly compared in the discrimination experiment. Both species showed a good discrimination ability: bumblebees could discriminate between all 6 pairs of figures, while honeybees did not distinguish an empty circle from an empty triangle. In the preference tests, the three different figures of each generalization test were compared. The segment-made figures were never the preferred ones, either in bumblebees or in honeybees. While bumblebees showed high variability in the preferred figures, honeybees expressed strong preference for figures other than the segment-made ones, with high homogeneity. This is likely to be the reason for the honeybees difficulty in choosing them in the generalization experiment.

12-084

OLFACTORY MEMORY IN THE HONEYBEE : PERSISTENCE AND RESISTANCE TO EXTINCTION
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In the honeybee, olfactory learning has been widely studied using the proboscis extension bioassay in restrained individuals, where an odour (conditioned stimulus - CS) is associated with a sugar reward (unconditioned stimulus - US). Based on this bioassay, we investigated the persistence of the memory trace along time, and its resistance to the interference of contradictory information. After a single CS (pure linalool)-US (30 % sucrose solution) pairing, 55 % of the bees showed lifetime conditioned response to the CS, when presented once. The unrewarded presentation of a previously rewarded CS induces an extinction of the conditioned responses. Five successive trials are sufficient to bring the bees' responses back to a spontaneous response level, independently of the inter-trial duration. This decrease was found to be possibly transient since a recovery of the level of response could be observed at a later stage, as shown by varying the durations between the conditioning and extinction trials (1 or 10 min), between the extinction trials (1 or 10 min), and between the extinction trials and a later trial (1, 15 min, 1 or 24 h). Thus, up to 70 % recovery could be obtained at the last trial. In addition, we carried out successive extinction phases alternating with recovery periods (1h) and/or with recall trials (rewarded presentations of the CS). The best conditions to maintain a high and stable level of conditioned responses were to apply both 1 h recovery period and 1 recall trial between the extinction phases. These experiments led us to tentatively define the temporal limits between a transient loss and a permanent impairment of the olfactory memory. These data are discussed with regard to the adaptative ability of honeybees to forage on floral sources with variable nectar reward.

12-086

THE INTENSITY AND SITE OF INFECTION OF *Aedes Aegypti* (DIPTERA: CULICIDAE) LARVAE WITH METACERCARIAE OF THE ENTOMOPATHOGENIC DIGENEAN *PLAGIORCHIS ELEGANS* (TREMATODA: PLAGIORCHIDAE) AFFECT THE ATTRACTIVENESS OF THEIR WATERS TO OVIPOSITING, CONSPECIFIC FEMALES
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A series of biological assays was conducted in the laboratory to assess the oviposition responses of gravid *Aedes aegypti* females to waters that had harboured conspecific larvae parasitized with the digenean *Plagiorchis elegans*. Infections were of various intensities and locations within the bodies of second, third and fourth instar larvae. Regardless of instar and location of infection, repellency of the waters to ovipositing females increased with the intensity of infection of the larvae. Similarly, waters derived from larvae with infection of the thorax and head tended to be more repellent than waters from larvae with abdominal infection only, regardless of instar and intensity of infection. Oviposition repellency was greatest in response to waters from second instar larvae infected with as few as three metacercariae. The number of eggs laid on these waters was only 61% of the distilled water controls. Similarly, second instars with infection of the thorax yielded waters which attracted only 50% as many eggs as controls. These data suggest that, under field conditions, sublethal infections of *Aedes aegypti* larvae with *Plagiorchis elegans* may reduce recruitment into the larval population.

12-087

SWARM FLIGHT BEHAVIOUR OF *RHAMMATOCERUS SCHISTOCERCOIDES* (REHN, 1906) IN MATO GROSSO, BRAZIL (ORTHOPTERA, ACRIDIDAE, GOMPHOCERINAE).

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A study of swarm flight behaviour in *Rhammatocerus schistocercoides* (Rehn, 1906), pest grasshopper of Mato Grosso State (Brazil), revealed a limited dispersal potential, in contrast to prior theories on their migration habits. The grasshoppers were monitored throughout the imaginal stage. In September, when their flight behaviour is maximal, several swarms were studied from morning to evening, with their behaviour noted at 1-min intervals. On average, their daily movements did not exceed a few hundred metres, even under optimal thermal conditions. The longest distance covered in one day was 2.8 km. Swarms showed standard rolling-type flight behaviour, and many grasshoppers remained at roost as others took flight. The swarms flew at quite low altitudes, not higher than 5-10 m. Flight directions were mainly determined by the direction of prevailing winds. These corresponded to local migrations that enabled the swarms to fully explore their environment (savanna regions with shrubs and trees) during the dry season when conditions are generally unfavourable. A predominant theory over the last 10 years holds that these grasshoppers regularly migrate eastwards; this now seems quite unlikely.

Since the species is much less mobile than previously supposed, a preventive control strategy aimed at detecting and destroying hopper bands during the rainy season could be possible. Control operations conducted jointly with producers' groups should significantly reduce the grasshoppers populations.

12-089

TERRITORIALITY AND CLEPTOPARASITISM IN A POPULATION OF *AMMOPHILA SABULOSA* (HYMENOPTERA: SPHECIDAE) IN ALTA VALTELLINA (NORTHERN ITALY)

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This study has been carried out at Le Prese (Alta Valtellina, Sondrio province; Lombardia), in the period June-August from 1993 to 1995. The behaviour of about 400 marked *Ammophila* has been recorded. They were followed every day from 08.30 a.m. to 07.00 p.m.. All nests digged by the females have been labeled and numbered, aiming at acquiring more informations about species territoriality.

Some cases of cleptoparasitism have been observed between females nesting in neighbouring areas. This behaviour has been performed by a "thief" female soon after provisioning, nest closing and leaving of the "owner" female.

12-088

THE BEHAVIOR OF GERMAN COCKROACH, *Blattella germanica*, TO LOCATE A NEW HIDING PLACE

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The behavior of finding out a new hiding place by German cockroach, *Blattella germanica*, was investigated experimentally under laboratory condition.

As an experimental setting, a large container (64x38x15 cm) set with feed and drinking water at the center was connected with a small box (15x11x8 cm) as a hiding site. A group composed of 25 cockroaches were released into a small box and acclimated to the experimental environment for a period of seven days. After adaptation, a new small box was set up as a new hiding place at the opposite side of the container, and the number of individuals were counted every day.

In experiments using separate groups (populations composed of only males, gravid females, or ungravid females), it was observed that the adult male groups disseminated evenly to the whole area of the container and also immigrated into the new hiding site more rapidly than gravid female or ungravid female groups.

In case of mixed groups of adult males and gravid or ungravid females, the former migrated into the new hiding place more quickly than the latter. However, spreading behavior of males seemed to be influenced by the presence of gravid or ungravid females, because they were not so active as observed in the test employing separate male groups.

12-090

THE IMPORTANCE OF BODY SIZE IN THE COCKROACH WASP *AMPULEX COMPRESSA* (F.) (HYMENOPTERA; SPHECIDAE; AMPULICINAE)

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An important relation in parasitoid behavioural ecology is that between size and fitness. Differences in body sizes are often assumed to affect the reproductive success of females more than that of males. Size related components of fitness take place in a variety of direct and indirect forms. Some of them were studied in *A. compressa*, an idiobiont, solitary parasitoid of several species of blattid cockroaches. The wasp's body size is determined by host size and may differ up to a factor 5.

No correlation was found between size and fitness, when fecundity and longevity were used as fitness parameters. Beyond fecundity and longevity the reproductive success of *A. compressa* females was also measured by investigating the influences of size and ownership-status on the outcome of owner-intruder contests for oviposition sites. Both body and status influence outcomes of contests: larger females nearly always win, but after oviposition, outcome is modified in favour of owners also when intruders are much larger.

It is unlikely that males have direct contests for virgin female resources. The benefits of being larger could not be established in males and they also do apparently not suffer from being small.

12-091

SWARMING IN BLOOD-SUCKING MOSQUITOES (DIPTERA,CULICIDAE)- RITUAL OR COMPETITION?

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The emergence, sexual maturation, swarming and mating behavior were studied in natural population of *Aedes communis* DeGeer. The duration of male sexual maturation period was estimated by the content of proteins in the accessory gland secret. During this period the quantity of proteins increased 2.5 times and reached maximum on the 5th day after male emergence when the swarming behavior started. Simultaneously the appearance of inseminated females was observed. This shows the correlation between swarming and mating in *A.communis*. Two types of males were revealed in the population: large males with longer wings and higher protein content in accessory gland secret and small ones with shorter wings and lower protein content. Only large males were observed to swarm and mate. The mating however rarely occurred in swarms. Moreover, only a few swarming mosquitoes were attracted to virgin females flying across the swarm. Thus during swarming the majority of males was not ready to couple. Usually the males left the swarm after 6-10 min of swarming and started a free flight which could result in 1) joining another swarm or 2) landing or 3) mating. The data suggest that swarming behavior seems to be necessary for selection of more viable males and serves for their sexual excitement.

12-093

TEMPORAL PATTERNS OF PRESENCE AT MICROHABITATS AND LOCOMOTION FOR THE GERMAN COCKROACH, *BLATTELLA GERMANICA*, IN SEMI-NATURAL CONDITIONS

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A genetic mutant, orange body (ob), of the German cockroach, *Blattella germanica*, was observed continuously in semi-natural conditions through video taping and data processing by computers. In a rearing cage, four microhabitats were provided to represent important behavior of the test insect, resting, feeding, drinking, and communicating with other individuals. Adult males from the same maternal origin were randomly selected, and presence at the microhabitats was individually observed for 4 - 6 days continuously under the light condition of L12:D12. Relatively large variations were observed among individuals in the daily temporal patterns of presence and visiting at the microhabitats. In average they stayed at the place for resting for the longest duration, about 50 percent of the total observation time, while they were present at the other microhabitats for feeding, drinking, etc. for a short period, less than 3 percent of the observation time. Daily peaking time in presence and visiting was observed at some microhabitats. Although there were variations among all the individuals observed for the study, similar temporal patterns of presence and visiting at microhabitats were observed in some individuals from the same maternal origin when the continuously observed data for each individual were expressed in daily averages. The variations in presence at microhabitats were interpreted with principal component analysis, and the closeness in temporal patterns among different individuals was quantitatively demonstrated in this study.

12-092

ASYMMETRY AND HANDEDNESS IN CRADLE FORMATION OF SOME ATTELABID WEEVILS - AN ETHOLOGICAL APPROACH TO A BEHAVIOUR CONTROLLING SYSTEM (ATTELABIDAE).

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The formation of cradles, which are made of a young plant organ (e.g. a leaf cut and/or rolled), is a characteristic maternal habit among attelabid weevils. Concerning the rolling direction of the leaf, there are two types of cradles (i.e., dextral and sinistral ones). The two types form mirror images of each other and occur in the field commonly. The cradle formation process of several attelabid weevils (including *Phymatopoderus pavens* Voss and *Apodrus balteatus* Roelofs) was observed in the field and the laboratory. When a cradle was made, the rolled direction of the leaf was recorded for each weevil. Female weevils made both types of cradles, though the ratio of one rolling direction to the other was not always even. The side of the legs used at a certain stage of cradle formation of a cradle type was concretely fixed at left or right. These phenomena show that each female weevil can manipulate the legs of both sides, and also that in the process of a given cradle formation she behaves asymmetrically, right handed or left handed. A series of experiments were conducted for *P. pavens* to clarify the controlling mechanisms of this behavioural sequence and that of the laterality of the legs. The results suggest that the direction of asymmetrical manipulation of the legs is decided at an early stage after which it can not be reversed (e.g. form right handed to left handed).

12-094

DOMINANCE STATUS AND RECOGNITION OF THE OPPONENT IN MALE *GRYLLUS BIMACULATUS* (ORTHOPTERA : GRYLLIDAE) DYADIC CONFLICTS

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Both males of the dyad behave similarly at the beginning of their first encounter. In *Gryllus bimaculatus* dyads conflicts stable dominance was rapidly established. Along with successive encounters of the same males, aggressive behaviour was decreasing as the dominance status of each one was gradually settled. That process appeared weaker when one of the male is changed at each successive encounter. It has been concluded that the status of the tested male seemed established at the first session and was not modified by further encounters whether the opponent was familiar or not. One data did not demonstrate any time individual recognition but some kind of familiarity.

12-095

THE MYSTERY OF METHYL EUGENOL: II. LICKING BEHAVIOR OF THE CARAMBOLA FRUIT FLY, *BACTROCERA CARAMBOLAE*, ON A SPADIX OF *SPATHIHYLLUM CANNAEFOLIUM* (DIPTERA: TEPHRITIDAE - ARLES:ARACEAE)

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To test the hypothesis that naturally occurring methyl eugenol (ME) decreases the efficiency of the male annihilation technique using ME (Shelly, 1994), we observed the licking behavior of the carambola fruit fly, *Bactrocera carambolae*, on a spadix of *Spathiphyllum cannaefolium* which contains ME on the island of Java, Indonesia. Although distribution of ME-traps containing 1 g of poisoned ME placed 50 m from the *S. cannaefolium* community resulted in a decrease in number of males visiting the flowers, many flies were still attracted to the flowers. A series of outdoor screen cage observation showed that the degree of response to the flowers by the males which had licked ME on the flower on the previous day varied greatly among individuals, i.e. some did not respond at all while others licked at almost the same frequency or even more. Although the results seemed to support the hypothesis in part, additional observations are required to derive any conclusions.

12-096

UNUSUAL CALLING BEHAVIOUR IN *THERESIMIMA AMPELOPHAGA* BAYLE-BARELLE (LEPIDOPTERA: ZYGAEINIDAE) FEMALES

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Theresimima ampelophaga Bayle-Barelle females were found to perform unusual calling behaviour. At the time of active attraction males in field they never extruded the tip of the abdomen like almost all other lepidopterans. Instead of this females of this species curved dorsally their abdomen and remained in this posture for hours. Biotests with extracts of different parts of the female abdomen on conspecific males, further confirmed the presence of a pheromone source at the dorsal part of the abdomen. The presence of pheromone gland cells on this part of the female abdomen was also confirmed histologically.

Some data about other details of *T. ampelophaga* females calling behaviour are also presented.

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12-097

SEX PHEROMONAL STUDY OF THE TEA CLUSTER CATERPILLAR *ANDRACA BIPUNCTATA* WALKER. (LEPIDOPTERA: BOMBYCIDAE)

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Sex interactions between male and female of the tea cluster caterpillar *Andraca bipunctata* Walker (Lepidoptera: Bombycidae) are mediated primarily through pheromonal signals. Females produce a four-component pheromone blend in the pheromone gland, but only one major compound attracts males in the field. The major compound is identified as E11,E14-18:Ald by GC, GC-Mass and EAG method. The function of other three-component pheromones in the courtship of adult moths is also discussed.

12-098

APOSEMATIC SIGNALS IN MUTILLID WASPS (MUTILLIDAE, HYMENOPTERA)

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Mutillid wasps are solitary. Their larvae are parasitoids of the immatures of other insects, mainly of Aculeata (Specidae, Apidae). The female Mutillid wasps have a powerful sting, the integument is extremely thick and hard, and they are wingless. On account of searching host animals the females are exposed to manifold predators. The production of poison is not up to such kind of condition, and the poison production costs are high. Therefore, female Mutillid wasps produce multimodal aposematic signals before and during sting. In contrast to the optical signals, previous knowledge of the acoustical and the chemical signals is limited.

Mutillid wasps possess abdominal stridulatory organs between their 3rd and 4th tergites. The mechanical system is extreme damped. So the signal contains many harmonics. Because of the large wavelength of the fundamental frequency (relative to the tergite dimensions) the radiation of higher harmonics is proportional to the square of their frequency. Therefore, the spectrum is broad. The signals sound something like aposematic or startle signals of vertebrates. They are broadband noises similar to that sort of signals produced by hissing or rattling.

Additional to this it was found a third, possibly aposematic, signal: the chemical secretion in the mandibular gland. This gland contains a cocktail of 4-methyl-3-heptanone, and some pyrazines.

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Section 13

Social Insects

13-002

THE EVOLUTION OF SOLDIER CASTE IN APHIDS

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Soldiers in aphids are nymphs that defend their colonies against insect predators. Soldiers are characterized by sclerotized skin and enlarged legs or horns. These characters are used as weapons to counterattack predators. Aphid soldiers in some species do not molt after birth and constitute a caste distinct from normal nymphs. This dimorphism is determined before birth. Other species produce "monomorphic defensive nymphs", which are composed of all individuals at a specific nymphal stadium and can develop into adulthood. Monomorphic defenders have been considered as an evolutionary precursor of a sterile soldier caste.

To understand factors leading to a sterile soldier caste, this study focused on the function of monomorphic defenders of some pemphigid aphids. Aphid colonies producing monomorphic defenders were characterized by a high proportion of defenders, which usually accounted for more than 50% of all the members. This high proportion was due to the prolongation of the defensive nymphal stadium. Prolonging the defensive nymphal stadium is advantageous in increasing the proportion of defenders, but decreases the rate of population increase. A simple simulation model predicted that the evolution of dimorphism is more favored when the colony lasts longer and when predation pressure is higher. In addition to the consideration of these ecological factors, this study advances a hypothesis to explain how the soldier morph originated in the aphid populations.

13-001

HORMONAL ASPECTS OF PHASE POLYMORPHISM IN LOCUSTS; ARE THERE SIMILARITIES TO CASTE POLYMORPHISM IN SOCIAL INSECTS?

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Locust show density-dependent continuous phase polymorphism. They appear in two forms, termed phases, "gregarious" and "solitary" which differ in many characteristics. There are intermediates between the two phases and phase transformation, induced by changes of density, is not instar or stage specific.

It was generally accepted that high activity of the corpora allata and high juvenile hormone (JH) titres induce the solitary phase. JH indeed induces certain solitary phase characteristics, the green solitary colour being the best demonstrated example. However, some phase characteristics are not affected by the JH and JH even shifts some other phase characteristics toward gregarious direction. The possible roles of neurohormones in phase change have been neglected, probably because of the overestimated importance of the JH. Recent research started to close this gap; phase-dependent differential responses to adipokinetic hormones were demonstrated and a dark colour-inducing neurohormone affecting phase colour polymorphism was discovered. Although pheromonal effects on phase change are intensively reinvestigated, the endocrine relations of aggregating pheromonal effects have not yet been studied. Nowadays the endocrine aspects of locust phase polymorphism seems to be more complex than it was previously assumed and this conclusion may be instructive also for research on the endocrine basis of caste polymorphism in social insects.

13-003

ORIGIN AND EVOLUTION OF IRREVERSIBLE DECISION POINTS IN TERMITE DEVELOPMENT

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In termite societies, every individual sooner or later reaches decision points where it faces alternative developmental pathways: e.g., for a termitid larva, following the sexual (wing-budded nymph, alate) or neuter (worker, soldier) developmental pathway; for a worker, moulting to the next worker stage or to a presoldier, etc. The most remarkable decision points are those leading to well-defined morphological castes: soldiers and workers. The omnipresence of the fully sterile soldier caste in extant termites makes investigations into its origin difficult. By contrast, the diversity of caste patterns regarding the individuals performing worker tasks allows a comparative approach to the origin of the true workers, which evolved independently of soldiers to constitute a second eusocial caste in the higher termites. The physiological bases of worker differentiation can be traced back to roaches and are widely expressed, reversibly, during regressive moults from nymphs to pseudergates in lower termites such as *Neotermes* or *Proterhinotermes*. Sterile soldiers and true workers are both characterized by the irreversibility of the developmental decisions leading to these castes. Their remarkable diversification suggests that the irreversibility of developmental choices is a threshold of major evolutionary significance. An overview of termite caste systems suggests that true workers evolved in populous colonies inhabiting large logs (like some present-day Rhinotermitidae) and diversified later on to develop complex foraging strategies.

13-004

STORED PROTEINS IN CASTE AND COLONY DEVELOPMENT IN ANTS

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Storage proteins are important during development in ants, as they are in most insects, allowing temporally distinct periods of eating and metamorphosis. In addition, adult ants in some taxa are able to store the same kinds of proteins normally accumulated during development. These proteins stores can then serve as an amino source for intensive protein synthesis by the storer herself. Or, it can be transferred to nestmates, profoundly affecting their nutritional state.

Claustrally-founding queens accumulate large quantities of protein between eclosion and the mating flight. These reserves, along with fats, are depleted while the first brood of minor workers is reared. In ants today, stored protein is associated with the claustral mode of colony founding. The ability to express the predominantly larval genes for storage protein synthesis in the adult stage could have been a key to the evolution of claustral founding.

Workers in some species of *Camponotus* are capable of storing protein over winter. I hypothesize that reserves are mobilized in the spring and transferred to larvae to support growth, and to the queen to support higher rates of egg production. Workers of other Formicidae species, such as *Formica* and perhaps *Prenolepis*, appear to store large quantities of fat but little storage protein in their abdominal fat bodies. The role of proteins stored elsewhere in the body in supporting seasonal egg laying and brood production should be evaluated. Stored protein, along with other reserves, could provide the basis for seasonal production of exceptionally rich larval food. In ants, distribution of this rich food would be regulated temporally, with larvae present at a restricted time of year receiving the mixture. As in honeybees, exposure to a rich diet could play a role in caste determination. Distribution of royal jelly in honey bees, in contrast, is regulated spatially, with workers giving the substance only to larvae in specially constructed cells.

13-006

HORMONAL CONTROL OF HONEY BEE CASTE DEVELOPMENT

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Juvenile hormone (JH) has long been known as a key factor in caste morphogenesis of social insects. In the honey bee, caste-specific JH titers in the fifth larval instar are produced by differential activity of the corpora allata (CA), and it is particularly during the first half of this instar that JH production in CA is much higher in queen than in worker larvae.

Using a radiochemical in vitro assay to monitor CA activity we attempted to identify factors that modulate JH synthesis in honey bee larvae. Extracts prepared from larval brains were found to exhibit both stimulatory and inhibitory effects. Among these factors we could single out two biogenic amines, octopamine and serotonin that stimulated CA activity in worker larvae. The action of these biogenic amines appears to be mediated by cAMP as second messenger. In the feeding phase, however, JH synthesis in worker larvae could only be stimulated to a limited extent and never be brought to the levels measured for queen larvae. This appears to be due to a rate-limited conversion of methyl farnesoate in a terminal step of JH biosynthesis.

The mode of action of JH in honey bee caste differentiation can best be described as pleiotropic. Probably one of the most important steps is an interendocrine activation of the prothoracic glands to install a queen-specific program of ecdysteroid synthesis. Partially this involves a direct pathway, as shown by in vitro stimulation of prothoracic glands by methoprene. In the gonads, the organ exhibiting the most divergent caste-specific morphology, juvenile hormone application to fourth instar worker larvae induces full differentiation of germ cells and follicle epithelial cells, and thus prevents initiation of a cell death program normally occurring in the fifth instar in most of the ovarioles. Later in the fifth instar, ecdysteroids then come to play important roles in caste development. In vitro experiments demonstrated that caste-specific expression patterns of two major proteins in the larval ovary are regulated by methyl farnesoate. As one of these is a heat shock protein, generalized secondary effects of ecdysteroids on ovary morphogenesis are to be expected.

13-005

FOOD, FEEDING AND CASTE DIFFERENTIATION IN BUMBLEBEES

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Caste polymorphism in bumblebees is like in all social Hymenoptera related to differences in larval nutrition. In *Bombus terrestris* caste specific development is in the first instance determined by queen pheromones¹. This paper deals with whether or not, differences in feeding behaviour or in larval food composition play a role in caste determination and differentiation in *B. terrestris*.

Comparisons of food samples collected from prospective worker, queen and male larvae revealed no differences in pollen, protein and carbohydrate concentration. Therefore caste differentiation in *B. terrestris* is not influenced by qualitative differences in larval food. The glandular secretions added by feeding workers, are salivary in function rather than food additives playing a major part in caste differentiation.

The distinct difference in the feeding frequency for prospective queen and worker larvae could account for caste specific development. In *B. rufocinctus* adult size is the outcome of differential growth rates and silk production, as a result of differences in feeding frequency². In-vitro feeding experiments show that *B. terrestris* larvae stop feeding and prepare for pupation, even when growth is not restricted by the silk production or if the feeding frequency is increased dramatically.

In experimental set-ups starved larvae were provisioned by workers significantly earlier and more often than control larvae. This suggests that larvae themselves influence the feeding frequency as a result of their nutritional status, and that feeding rate is not imposed on them by the workers. The fact that feeding frequencies differ for worker and queen larvae suggests that they have different nutritional needs.

Our results show that worker and queen larvae have alternative developmental programs, switched on or off at an early age by queen pheromones, and that caste development is regulated by this program and the amount of nutrition.

¹ Röseler, P.F. (1970) Unterschiede in der Kastendetermination zwischen den Hummelarten *Bombus hypnorum* und *Bombus terrestris*. Z. Naturforsch. 25b: 543-548

² Plowright, R.C. and S.C. Jay (1977) On the size determination of bumble bee castes (Hymenoptera: Apidae) Can. J. Zool. 55: 1133-1138

13-007

THE EFFECT OF SOCIAL ENVIRONMENT IN THE COLONY ON CASTE DETERMINATION AND JH SYNTHESIS IN *BOMBUS TERRESTRIS* LARVAE

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Queen production in *B. terrestris* occurs late in colony development and is correlated with the onset of the competition phase between workers and their mother queen. To understand the impact of the existing queen on caste determination, we compared the development and JH production by the corpora allata (CA) in queen and worker prospective larvae. This is the first time that JH production has been measured in bumble bee larvae.

In queen larvae the duration of all 4 instars was longer, and their head width at the third and fourth instars was significantly larger than worker larvae. In addition worker larvae were characterized by a constant low JH biosynthesis that did not surpass 5 pmolJH/h/pair. In queen larvae JH production was higher (peaking at 45 pmolJH/h/pair) starting from the end of the second instar and throughout most of the third instar.

The effect of the social environment on caste determination was examined by transplanting egg cups from young "donor" colonies into "host" colonies either before or during the competition phase. When the resulting larvae were 5 days old, they were weighed, their larval instar was determined according to head width, and their CA activity was measured. Larvae that developed before the competition phase had accelerated development as compared to those reared after this phase. They were heavier (57.3±27.8mg and 34±13.8mg respectively) and greater proportions were at an advanced developmental stage: 40% at the 2nd instar, 40% at the 3rd instar and 20% at the 4th instar, as compared to 70% at the 2nd instar, 25% at the 3rd instar and 5% at the 4th instar for larvae that developed in nests during the competition phase.

CA activity in these larvae was characterized by two distinct levels of JH synthesis parallel to the two different castes. Larvae between 2-5 days old when the competition phase began, had a low JH synthesis rate of 5.7±2.8 pmol/h/pair and eventually developed into workers. Larvae younger than 2 days when competition began, had a significantly higher JH synthesis rate of 20.7±5.6 pmol/h/pair and developed into queens.

In conclusion, queen development involves longer developmental time of each larval instar and is associated with high JH production, both of which are apparently affected by the social status of the queen in the colony.

13-008

THE STOMOGASTRIC NERVOUS SYSTEM AND THE NEUROENDOCRINE AXIS IN HONEY BEE DEVELOPMENT

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Anatomical and morphological studies in insects show the existence of direct connections between the stomogastric nervous system (SNS) and the digestive and endocrine systems. Compared to the central nervous system (CNS), the SNS of the honey bee has received little attention with the most recent studies dating back to the 40's. We therefore decided to reevaluate the structural organization of the stomogastric NS using modern histological methods, and related it to the function of the neuroendocrine system.

During the fifth larval instar we observed the frontal ganglion, the center of the stomogastric NS, to be more developed than in the pupal phase, the same being true for the connections of the recurrens nerve with the brain and oesophagus. The hypocerebral ganglion presents short branches along its lateral margins in close connection with the digestive system. We detected in the brain of this larvae 22 neurons expressing prothoracicotropic hormone (PTTH, a regulator of postembryonic development in insect), distributed in 5 clusters. These were in the subesophageal ganglion, in the antennal rudiments and also in the corpora allata (CA) and corpora cardiaca (CC). Only a single pair of neurons expressed PTTH in white-eyed pupae. Similar results were obtained when we immunostained serotonin (5-HT)-expressing neurons in larval honey bee CNS. The presence of varicosities and a small number of cells directly adjacent to the CA, oesophagus and in the wall of the aorta, point to a possible neuromodulator and neurohormonal mode of action, respectively, for PTTH and 5-HT of larvae and pupae of honey bees.

A lack of immunoreactive axons suggests a secondary role for the classical neuroendocrine axis, at the same time that immunoreactivity in the CC, within the CA and morphological alterations in the frontal ganglion and recurrent nerve in the 5th instar, appear to indicate that the SNS has a much greater role in developmental control of honey bee than was previously thought.

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13-010

PHYLOGENY, BIOGEOGRAPHY, AND SYMBIOTIC FLAGELLATE COMPOSITION OF *RETICULITERMES* SPI (ISOPTERA: RHINOTERMITIDAE) AROUND THE JAPAN ARCHIPELAGO

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Lower termites possess symbiotic flagellates that take an important part in cellulose digestion of their hosts. Species composition of the symbiotic flagellates is generally known to be specific to their host species or regional populations.

In this study, the flagellate species composition was investigated of *Reticulitermes* spp. colonies around the Japan Archipelago. Phylogeny of these host species and regional populations was also estimated from the partial sequences (591bp) of the mitochondrial gene (cytochrome oxidase subunit II: COII), using neighbour-joining method.

The general trend of the species- or regional population-specificity of the flagellate composition was observed. The patterns of the similarity show well correspondence to the geographical distribution of the hosts. The largest difference of the composition was detected across the Watase Line, biogeographical border between paleoarctic region and oriental region in the area. Host species around the Japan Archipelago were divided into two monophyletic groups from the analysis of COII sequences. The largest genetic break that is supported by high bootstraps was detected across the Watase Line. The phylogeny of the hosts shows good accordance to its patterns of geographical distribution except a few species. Host phylogeny and flagellate species composition also show considerable correspondence and a large effect of host phylogeny on the flagellate composition is suggested. However, the mapping of the states of each flagellate (presence or absence) onto the host phylogeny does not result in good congruence in some flagellate species. It was possible that these flagellates were horizontally transmitted between sympatric host lineage.

13-009

SIMILAR TRENDS IN HYMENOPTERA EUSOCIALITY (MELIPONINAE, BOMBINI, EPIPONINI)

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Bionomical aspects of *Bombus atratus* and stingless bees (e.g. laying workers) are focused under insights derived from studies on the swarm-founding Polistinae wasps in which the intriguing presence of intermediate females is quite common. They were first evidenced by Richards & Richards (1951) as he recorded uninseminated but ovary-developed wasps in colonies of several Epiponini taxa. Although their role remains largely unknown there are direct observations on their frequent egg-laying and oophagy in *Protopolybia exigua* (Simões, 1977) and *P. acutiscutis* (Naumann, 1970). Presently, ongoing morphological studies on caste differences have been providing interesting results on their distribution, morphological and physiological identities, etc. In this connexion, their presence seem to be negatively correlated to inter-caste differences. They are invariably absent under stronger queen-worker dimorphism (*Agelaia*, *Apoica*), invariably frequent at inconspicuous castes differences (*Pseudopolybia*, *Parachartergus*, *Brachygastra*), and cyclically present (Noll, 1995) when caste differences are slighter (*Protopolybia*, *Polybia occidentalis*). In addition, intermediates can be transitional in *Parachartergus*, that is, after mating they can reach the queen status (S. Mateus, unpub.) while in other taxa their consistent morphological characteristics seemingly warrant them their fixed intermediate characteristics (Shima et al., in press). On these grounds, the intermediate concept is expanded to other inclose groups, namely the laying workers of stingless bees of which the produced trophic eggs constitute important part of queen's nutrition, and the two kinds of laying females that precisely characterize parts of the unusual colony-cyclic of the neotropical *Bombus atratus* by eventually producing males and females.

13-011

A NEW GENUS OF DOLICHODERINAE (HYMENOPTERA: FORMICIDAE) IN DOMINICAN AMBER, WITH A REAPPRAISAL OF THE PHYLOGENY OF THE SUBFAMILY AND A RECONSTRUCTION OF IMMATURE TRANSPORT

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We describe a new genus of Dolichoderinae ants, based on workers, and larvae, preserved along with eggs and pupae of the same species and four other insects, in one piece of amber ("Jorge Caridad") found in Palo Quemado Mine, near Santiago, Cordillera Septentrional, Republica Dominicana (Oligocene to lower Miocene). We discuss the affinities of this genus with the other extant and fossil Dolichoderinae and offer an interpretation of the fossilization event, in special the immature transport by the ant workers.

Our analysis of 64 characters of workers, gynes, males, and larvae of 23 extant Dolichoderinae genera allow us to propose a new arrangement and to justify the description of the new genus.

To each ant worker we were able to assign a group of eggs (3 groups), a mature larva or a group of young larvae (31 larvae in total), or a pupa (18 individuals). This suggests that a group of "nurses" was trapped in resin, while carrying immatures away from the imminent peril.

The description of an extinct genus of Dolichoderinae strengthens the notion that this subfamily seems to be retracting from its former dominant status. If we consider the very closely related Aneuretinae plus Dolichoderinae against Myrmicinae, this impression becomes even stronger, as only *Aneuretus* lives today, and the other four aneuretine genera are extinct.

13-012

QUEEN MATING FREQUENCY AND WORKER REPRODUCTION IN THE WASP *DOLICHOVESPULA ARENARIA*F. L. W. Ratnieks, J. J. Boomsma¹

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Theoretical studies indicate that worker policing, the mutual inhibition of reproduction by workers, will be most strongly favored by natural selection in single-queen hymenopteran societies when the effective mating frequency of queens is greater than two. Comparative studies of mating frequency and worker production of males in bees (Apidae) support this prediction. Species with multiple-mated queens (*Apis*) produce few workers' sons in queenright colonies. Species with single-mated queens (*Bombus*, *Meliponini*) produce many workers' sons in queenright colonies.

The vespine wasps provide an ideal group for studying the effect of variation in queen mating frequency on worker reproduction across a group of species with similar life cycle and general biology. Ross (1986, *Nature* 323:798-800) has demonstrated queen mating frequency greater than two and absence of worker production of males in two N. American *Vespula* species. Here we report that in *Dolichovespula arenaria* queen mating frequency is close to one and workers frequently produce males in queenright colonies. Thus, the data on reproduction in vespines are in good agreement with the predictions of worker policing theory.

Allozyme analysis of two variable loci (HK, PGD) was carried out on males, workers, and the mother queen (where present) in 28 *D. arenaria* colonies collected during the reproductive phase of the life cycle near Berkeley, California. By analysis of workers double mating was confirmed in 7/28 queens. The probability of detecting a second mating was c. 0.7. Worker production of a large proportion of adult males (all from eggs laid prior to queen death) occurred in approximately half the colonies. Detailed analysis of one colony indicated that queen control of male production occurs but is incomplete. In this colony, most males reared in the centre of the nest (combs 3, 4) were the queen's sons and most in the upper and lower parts (combs 1, 2, 5) were workers' sons. Queen control gradually diminished with time until the queen produced no males, presumably because she was killed by the workers.

13-013

SEX ALLOCATION AND PARENTAGE IN COLONIES OF THE FACULTATIVELY POLYGYNOUS ANT *MYRMICA TAHOENSIS* (HYMENOPTERA: FORMICIDAE)

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In eusocial Hymenoptera, workers are often related asymmetrically to the male and female sexuals they rear. The magnitude of this relatedness asymmetry is predicted to depend on both queen number and the mating frequency of queens. When the number of related queens, or the number of times these queens mate, vary across nests in a population workers are predicted to specialize on producing the sex to which they are relatively most closely related. This hypothesis is one of several that might explain the widespread occurrence of split sex ratios (colonies specializing on the production of males or females) in ants.

In this study, I used variation at highly polymorphic microsatellite DNA loci to assess genetic relatedness in male- and female-producing colonies of the facultatively polygynous ant *Myrmica tahoensis*. Relatedness levels (and the relatedness asymmetry) were highest in colonies of *M. tahoensis* that produced female sexuals, providing support for the Relative Relatedness Asymmetry hypothesis. An experimental manipulation suggests that workers use cues presented by larvae when making their sex-allocation decisions, and that they can bias sex ratios long after the eggs destined to become reproductive offspring are laid. Both fertile workers and queens produced males in *M. tahoensis* colonies, leading to potential conflicts over male parentage. In some colonies, workers produced all or most males, while queens appeared to dominate male-production in other colonies. The relative degree of worker reproduction did not appear to covary with genetic relatedness or other colony characteristics. Female sexuals were unanimously produced by a single, once-mated queen, even in colonies whose workers were the progeny of two or more queens.

13-014

SEX RATIO BIAS, WORKER-QUEEN CONFLICT AND SELECTIVE MALE BROOD DESTRUCTION IN ANTS

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New insights of the role of kin selection in the evolution of eusociality come from models centering on worker-queen conflicts and sex allocation in social Hymenoptera. Sterile workers may enhance their inclusive fitness by biasing sex ratios towards the sex they are relatively more related to as compared to the population average. Evidence to date largely supports worker control of sex allocation in several species. However, queens control the proportion of unfertilized (male) and fertilized (female) eggs they lay and are expected to lay a primary (egg) sex ratio of unity. Here we test whether workers manipulate queen-laid sex ratios to match their own colony-specific optimum by selectively eliminating male brood.

As predicted by theory *F. exsecta* colonies headed by a singly mated queen specialize in female production, whereas colonies headed by a multiply mated queen specialize in male production. In our two-year data set, primary sex ratios were close to unity in both classes of colonies. However, in colonies headed by a singly mated queen the proportion of females significantly increased between the egg and the adult stage, whereas no such changes occurred in colonies headed by a multiply mated queen. Thus workers raised their favoured female-biased sex ratios by selectively destroying male brood in colonies headed by a singly mated queen, whereas no male brood destruction occurred in colonies headed by a multiply mated queen opting for male biased or mixed sex ratios. Our results thus demonstrate for the first time that workers manipulate colony sex ratios by selectively destroying male brood in colonies headed by a singly mated queen to match their colony-specific optimum.

13-015

REPRODUCTIVE SKEW AND RELATEDNESS IN *LEPTOTHORAX* COLONIES (HYMENOPTERA: FORMICIDAE)

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The co-existence of several inseminated queens in ant societies causes conflicts about the partitioning of reproductive rights. In colonies of several formicoxenine ants, these conflicts break open and lead to the formation of reproductive dominance hierarchies by overt or ritualized aggression, in which only the top-ranking queen lays eggs (functional monogyny). In other species, all queens reproduce at more or less similar rates (polygyny). In a model proposed by Bourke and Heinze (1994), the extent of reproductive skew has been linked to various ecological parameters. Whereas apparent reproductive skew estimated from egg laying rates and ovarian development in *Leptothorax* and *Formicoxenus* closely fits the predictions made by this model, genetical data suggest that the factors underlying the sociogenetic structure of Formicoxenini societies are somewhat more complicated. Nestmate relatedness as calculated from allozyme frequencies is significantly lower than expected in apparent high-skew species, probably due to a frequent exchange of the highest-ranking queen. In contrast, in some, but not all populations of polygynous *Leptothorax*, in which queens seem to contribute equally to the egg-pile, nestmate relatedness is much higher than assumed from the number of queens present. A detailed analysis of field data and relatedness estimates in the Reichswald population of *L. acervorum* indicates that this might result from cyclical changes in queen number, caused by the adoption of new queens and colony budding, queen death or emigration, and a negative correlation between the number of adult queens and the production of female sexuals.

13-016

TESTING REPRODUCTIVE SKEW THEORY IN MULTIPLE-QUEEN ANTS USING MICROSATELLITES

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Vehrencamp (1983) and Reeve and Ratnieks (1993) developed a kin selection theory for the evolution of a stable reproductive skew in societies of multiple breeders. The theory predicts low skew (equitable sharing of sexual production) in facultatively polygynous ants (having multiple, egg-laying queens in some colonies). We are attempting to test this prediction in the ant *Leptothorax acervorum*. The method involves a parentage analysis using variation at seven polymorphic microsatellite DNA loci. Three of the loci were identified using information on the sequences of the flanking regions of microsatellites in two other ant species (a *Leptothorax* and a *Myrmica*); four of the loci were identified for the first time in *L. acervorum*. On average, each locus has 16 alleles (range, 2-44). Eight polygynous *L. acervorum* colonies were collected from a single field population. Almost all the dealate (colony) queens, along with a sample of the sexual progeny, were then typed at each of the seven loci. In total, 434 individuals were typed (on average, 5 colony queens, 17 young queens and 32 males per colony). Preliminary analysis of the data suggests that in some colonies a large proportion of progeny derive from mother queens that were not present on collection. This suggests high turnover of colony queens. In other colonies, preliminary analysis indicates mixed maternity of the progeny as expected if skew is low. Precise quantification of skew levels in order to test the theory awaits further analyses of these data.

13-018

MULTIFACETED PARENTAL INVESTMENT AND SEX RATIOS IN SOCIAL HYMENOPTERA.

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Theories of parental investment and sex ratio generally assume that a single resource (food) limits reproduction, but many organisms invest two or more qualitatively different types of resources in the production of offspring. Thus, these models fail to address observed variations in offspring size or correlations between environment and investment patterns. We examine the consequences of "multifaceted parental investment" (MFPI) for offspring provisioning and sex allocation when parental behavior is limited by more than one ecological variable. Under MFPI, the factor limiting parental fitness determines the currency of the optimization problem and colonies should adjust reproductive behavior to maximize fitness returns per unit of the limiting resource. In social Hymenoptera with long developmental times as sexual larvae and a short reproductive season as adults, resource abundance late in larval development cannot be used to increase the number of sexual offspring. In such colonies, the key limiting resource will be offspring number rather than food per offspring. Therefore, food surpluses should lead to larger offspring and an increase in the proportional investment in females. These predictions from MFPI are consistent with widely observed patterns of variation in offspring provisioning and sex allocation in the social Hymenoptera.

13-017

THE IMPORTANCE OF KIN-CONFLICT IN RESOURCE ALLOCATION DECISIONS IN ANTS

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All organisms must consider the life history trade-off between present and future reproduction. For social insects, this means investing a given amount of resources into both colony maintenance and production of sexual offspring. Because queens and workers have different relative relatedness values to male and female offspring in a colony, these parties are expected to attempt to maximize different fitness curves with respect to colony maintenance and sexual output. Furthermore, monogynous (singly-queened) and polygynous (multiply-queened) colonies have different relative relatedness asymmetries, and are expected to differ in sex allocation and resource allocation decisions (Pamilo, 1991). In a single population of the facultatively polygynous ant species, *Leptothorax acervorum*, monogynous colonies were found to invest more heavily in the production of sexuals than polygynous colonies, which favoured investment in new workers. As colony size (and therefore probability of survival) increased, monogynous colonies, but not polygynous colonies invested increasingly in sexuals. All polygynous colonies invested heavily in new males, and the larger colonies invested decreasingly in new queens. These results suggest that polygynous colonies reproduce partly by budding, and their daughter colonies may experience local resource competition. Environmental stress and/or habitat saturation may cause polygynous colonies to favour reproduction through budding (Keller, 1995) and both queens and workers from small monogynous colonies to invest in colony maintenance.

13-019

REPRODUCTIVE STRUCTURE IN PRE-EMERGENCE COLONIES OF THE SOCIAL WASP, *POLISTES CAROLINUS*

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We studied reproductive conflicts in pre-emergence colonies of *Polistes carolinus* in Texas, USA. We combined census data on natural wild colonies from initiation to the end of the study with periodic videotaping and assessment of relatedness and parentage from DNA microsatellites. With these data we test hypothesis about relative roles of order of arrival, size, and relatedness in reproductive conflict among foundresses. We further evaluate predictions of the skew hypothesis in an experiment, where we removed eggs from nests, recorded behavior before and after the egg-removal, and genotyped the eggs removed.

Of the 28 colonies observed, 23 had more than 1 foundress at some point during the study period. None of the 5 single-queen nests survived till the end of the period. At the time of collection, 2 nests had a single queen, and 15 had multiple queens (2-8, mean 3.1). There was a certain amount of movement of foundresses between nests, especially when the foundresses came from the same natal nest.

The behavioral data consist of several hours (> 10 h/nest) of videotaped behavior. This allowed us to identify the behaviorally dominant female among the foundresses, as well as record many other types of behavior including work ratio and aggression levels. From the egg-removal experiment we determined whether aggression between foundresses increased, and if so, if it had anything to do with whose eggs were removed. We also determined if foundresses preferred their own progeny in feeding.

The genetic analysis was based on 8 highly variable microsatellite loci. All foundresses collected, the sperm in their spermatheca, and a sample of eggs and larvae from each nest were analyzed. With these data, each offspring was assigned to its mother. Preliminary results show that multiple foundresses were typically full sisters, which was somewhat unexpected based on previous allozyme results. Foundresses were also singly mated, and several females had reproduced in all multiple foundress nests.

13-020

EXPLANATIONS FOR HELPING BEHAVIOUR IN HOVER WASPS.

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Explanations for the evolution of worker behaviour in Hymenoptera have been dominated by the concepts of indirect fitness and genetic relatedness. In contrast, models of helping behaviour in cooperatively-breeding vertebrates emphasize the costs (ecological constraints on independent breeding), rather than the benefits of group living. Published work suggests that several features of the biology of stenogastrine wasps (Hymenoptera, Vespidae) more closely resemble cooperatively-breeding vertebrates than other social insects. For example, after foraging for a while on their natal nests, female offspring often appear to leave and reproduce elsewhere ('delayed dispersal'). Genetic relatedness between female nest-mates is lower than in any other primitively eusocial Hymenoptera. We use a mixture of observations and manipulative field experiments to approach three questions:

(1) Do non-dominant helpers monitor reproductive opportunities elsewhere, and will they abandon their roles as helpers when a high-quality opportunity arises?

(2) Do some females become helpers because they have low reproductive potential and cannot initiate nests and lay eggs on their own?

(3) What is the likely relative importance of benefits versus costs in the maintenance of helping behaviour in stenogastrines?

13-021

DEFENCE ALLOCATION IN CLONAL ANIMALS: SOLDIER INVESTMENT IN APHIDS OF THE GENUS *PEMPHIGUS*

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Aphids include the only highly social insects that are parthenogenetic. Kin-selected altruism ought to be rife in aphids, since all the members of an aphid colony, provided they are descended from a single individual and there have been no mutations, will be genetically identical. Yet reproductive altruism is rare: only about 1% (50/4,400) of aphid species have been reported to have soldiers. We will discuss this intriguing paradox in the context of a recent model of soldier investment in aphids.

This model (Stern & Foster, 1996) considers how selection might act on aphid clones to produce optimal investment in soldiers. It predicts that investment in soldiers will be favoured as birth rate decreases, instar duration increases, gall duration increases, predation rate increases, and clonal mixing decreases. We present here the results of recent experiments on soldier behaviour in five species of *Pemphigus* in the U.K., which provide the first tests of some of these predictions.

Stern, D.S. & Foster, W.A. (1996). The evolution of soldiers in aphids. *Biological Reviews* 71: 27-79.

13-022

FACTORS AFFECTING COMMUNAL GALL OCCUPATION IN THE APHID *TAMALIA COWENI* (HOMOPTERA: APHIDIDAE)

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Establishing plausible paths for the evolution of higher social behavior in the gall-making aphids requires the identification of stages intermediate to those of the free-living aphids and the gall-makers with a sterile soldier caste. One such stage is represented by the primitively social gall-maker *Tamalia coweni*: as it is facultatively communal, with a range of from one to ten foundresses occupying a gall, *T. coweni* offers the opportunity to test hypotheses concerning the origins and maintenance of group behavior.

I tested the effects of density on both the frequencies of gall-making and, more specifically, communal gall occupation. Both of these were significantly positively related to the number of foundresses per available gall-making site, although in no case were the available sites entirely occupied. Foundress survival to adulthood was significantly negatively related to the number of foundresses per gall, suggesting that communal gall occupation bears a fitness cost. Communal foundresses bore a further cost in reproductive fitness: the average biomass produced per foundress was negatively related to the mean number of foundresses per trial. However, the total number of foundresses surviving per gall was higher in two-foundress galls than in galls occupied by one or by three foundresses. If two foundresses occupying a gall are clone-mates, sharing a gall might thereby prove beneficial to the clone. Genetic markers are being developed to further elucidate the nature of the social relationships in *T. coweni*.

13-023

LIFE EXPECTANCY AND RISK-TAKING IN FORAGING HONEY BEE WORKERS

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The hypothesis that life expectancy is the main factor influencing risky foraging by worker honey bees was tested. We investigated whether diseased workers or workers with worn wings, and therefore with a shorter expected remaining life span, undertake foraging in poorer weather conditions than healthy workers or workers with unworn wings. Using a dynamic programming model we show that foraging honey bee workers should vary their foraging behaviour with age or disease status, foraging mainly under safe conditions when young or healthy with increased risky foraging with age or disease. Field observations of water collecting foragers confirm the model's predictions. Workers collecting water were captured in the middle of one cold and one warm day in April, May and July. Comparing each pair of days the ratio of individuals infected with *Nosema apis* spores was always higher on the cold day than on the warm day. We also found that there were more workers with worn wings collecting water on the cold days than on the warm days.

13-024

LIFE-HISTORY STRATEGIES IN ARBOREAL TERMITES FROM NEW GUINEA

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In coconut plantations on the north coast of New Guinea, three species of arboreal-nesting termites compete for food and space: *Nasutitermes princeps*, *N. novarumhebridarum* and *Microcerotermes biroi*. The three species build their nest on coconut tree trunks and forage for dead plant material on trees and on the ground. We investigated whether the three species display different life-history strategies. The number and morphology of the functional reproductives found in unmanipulated nests or obtained after orphaning experiments differ among species and suggest that *M. biroi* and *N. novarumhebridarum* are best adapted for colonizing trees by independent founders, whereas *N. princeps* would more easily spread by budding. Long-term field studies indeed showed that *Microcerotermes biroi* is very efficient at establishing new colonies in a virgin habitat, by means of nuptial flights. *Nasutitermes novarumhebridarum* also relies on nuptial flights to colonize new sites, but is more commonly associated with dead trees. By contrast, *N. princeps* seems to be a poorer colonizer, but established colonies are able to expand their territories by budding and form large unicolonial systems. Their fighting capacities allow *N. princeps* workers and soldiers to kill colonies of *M. biroi* and invade their territory. We suggest that differences in life-history strategies may account for the frequent co-occurrence of the three species in coconut plantations, in spite of the obvious competition between them.

13-026

PLASTICITY OF SOCIAL ORGANIZATION IN A FOREST ANT SPECIES

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The ant species *Myrmica punctiventris* displays remarkable variation in a number of life history characteristics. In a Vermont population, this ant has polydomous colonies, headed by a single-once-mated queen. There, coefficients of relatedness are very high, and sex ratios conform to predictions based on kin selection theory. By contrast, a population in New York has monodomous colonies headed by several queens, with lower relatedness coefficients and highly male-biased sex ratios. Furthermore, the New York population shows evidence of inbreeding and population subdivision. To understand the ecological factors that might produce such different patterns of social organization, we conducted an experiment on the Vermont population that added food and/or nest sites in a full factorial design. We found that the food supplementation had dramatic and consistent effects on the patterns of polydomy, polygyny, relatedness coefficients, sex ratios, and levels of selection. The nest site supplementation treatment also affected some aspects of social organization, and an interaction between the treatments was important for others. Thus our data show that social organization is ecologically labile. Studies of colony organization must take into account the environment in which those colonies occur.

13-025

SOCIAL STRUCTURE OF *LEPTOTHORAX* SP. A: INFLUENCE OF GENETIC COMPOSITION, AGGRESSION AND FERTILITY OF INDIVIDUAL QUEENS

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The North American formicoxenine ant species *Leptothorax* sp. A was thought to be strictly functionally monogynous (i.e. although several queens are present only one reproduces) and the observed fighting among intermorph (wingless) queens after hibernation leads to budding of the colonies and secures functional monogyny. We report a second fighting period during the annual colony cycle after the adoption of young mated queens into their mother colony, which does not lead to budding. Instead young queens are integrated into the existing hierarchy. Aggression can continue very weakly throughout the year, indicating that it is necessary for the inhibition of subordinates, as experiments suggest a lack of pheromonal inhibition. Furthermore, sampling data reveal a high number of monogynous colonies and dissecting data of queens from 35 polygynous colonies suggest, that colony organization in this species is far more diverse. Colonies can either be mono-, fet. mono- or polygynous. As models on ESS predict covariance of relatedness and asymmetry of reproduction and relatedness data show that multi-queen colonies in this species can be mother/daughter, daughter/daughter and/or mother/daughter/-aunt/nieces associations, these would be ultimate factors for *Leptothorax* sp. A in the process of deciding to fight and become fet. monogynous or not to fight and become polygynous. Among the proximate factors, size, weight, morphology and insemination have no influence on dominance ranking in queens. Individual fertility of a queen is shown to have a substantial influence. Experiments indicate that developing ovaries of a queen can be sensed by workers since they exhibit a preference for the most fertile queen and not the one having been dominant following aggression. This also indicates that aggression is energetically costly for the individual queen. Giving a queen a head start in ovary development before the others leads to budding of the colonies without aggressive behavior and the fertile queen is surrounded by the most worker and brood force. The conclusive prediction is that depending on the relatedness composition of a colony (influenced by the adoption of related queens and/or frequent superseding of high ranking queens) aggressive behavior can occur and if so, influence individual fertility of a queen by its energetic impact and thus, leading to differential reproduction and functional monogyny.

13-027

WEAKLY SPECIALIZED QUEENS AND THE ABILITY OF WORKERS TO MATE AND REPRODUCE: LIFE HISTORY OF THE PONERINE ANT *HARPEGNATHOS SALTATOR*

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In the indian ant *H. saltator*, new colonies are established by single queens. Young workers are able to mate with males from their own nest, but they remain sterile in presence of the foundress. However, when the latter dies, several mated workers become the reproductives (= gamergates). Since new workers mate annually, an orphaned colony can thus survive for some years. Alate queens continue to be produced in gamergate colonies, and they disperse and outbreed. Unlike other ponerine ants, the nests of *H. saltator* become extremely complex as they grow larger, and represent a valuable resource (Peeters & Hölldobler 1995). Colony emigration and eventual fission are thus unlikely to occur, and flying queens remain essential to start new colonies. *H. saltator* has small colonies (average 51 workers), but its life history resembles that of many termites - valuable nests are inherited by neotenic (wingless) reproductives, and outbreeding alternates with inbreeding. *H. saltator* is an interesting contrast to the several ponerine ants where queens have disappeared permanently. In these, colonies with gamergates can multiply through fission (the nests are often simple or short-lived), and the workers are thus totipotent. It appears that the weakly specialized queens (no claustral foundation; low fecundity; short lifespan) characteristic of morphologically "primitive" ants are selected against in various ecological contexts, but in *H. saltator* their production remains adaptive because colonies do not fission.

Peeters C. & B. Hölldobler (1995) *PNAS* 92: 10977-10979.

13-028

EVOLUTION OF EUSOCIALITY IN TERMITES: ROLE OF ALTRICIAL OFFSPRING

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There are obvious gradients in the maturity and relative size of cockroaches at hatch, ranging from immature and helpless (altricial) to complete independence from adults (precocial). The subsocial, wood feeding Cryptocercus punctulatus represents the extreme of altricial development among cockroaches. 1st instar nymphs lack compound eyes; eye pigment does not develop until the 2nd instar. The cuticle is pale and thin, with internal organs clearly visible through the surface of the abdomen. Gut symbionts are not established until the 3rd instar, making young nymphs dependent on adults for food. 1st instars are small, averaging just .06% of their final adult dry weight. The small size of neonates is associated with the production of small eggs by the female; length of the terminal oocyte is 5% of adult length, contrasting with the 9-16% exhibited by 6 other species of oviparous cockroaches. It is proposed that the subsocial ancestor of termites exhibited a suite of life history characteristics that included altricial development of offspring. By producing small eggs from which hatch dependent offspring, the female avoids incurring a large metabolic expense prior to hatch. A portion of reproductive investment is postponed until the post-embryonic stage, allowing some of the cost to be shared with other family members: the male and older offspring.

13-030

LIFE HISTORY STRATEGIES IN SWARM-FOUNDING WASPS

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Swarm-founding Polistinae are the dominant social wasps in the tropics. To understand the reasons for their success we need to analyze life history variation within the group. To date there have been virtually no studies directly addressing life histories in these wasps. For a handful of species, however, data do exist that can be used to estimate colony age and size at maturity, frequency of reproduction, seasonal timing of reproduction, and swarm size and number. I draw on these data to outline some of the variables and constraints involved and to begin to discern patterns in the life history strategies and trade-offs adopted by these wasps. Among the issues addressed are the following. (1) Because its 'offspring' are swarms of workers and queens, the swarm-founding colony has a potentially unlimited flexibility to adjust the trade-off between offspring size and number. A colony of swarm-founders can opt to produce a few large swarms or a larger number of smaller ones. These options are examined in light of the recent finding for *Polybia* that the per capita rate of production of offspring rises with swarm size. (2) In addition to the usual components of a species' life history, social wasps make decisions about the size of the nest. Because brood are reared singly in cells, the size of the brood population the colony can rear is tightly constrained by the number of cells in the nest. How large a nest to build is an especially important decision for swarm-founding wasps because the swarm completes the nest in the first 5-7 days of the colony's existence and then lives within its limits for the next several weeks. The fact that the size of the nest is tightly correlated with the number of wasps in the swarm suggests strong selection to optimize nest size. I examine nest size in species for which data exist in an attempt to identify what some of these selective pressures may be.

13-029

GEOGRAPHIC VARIATION IN SOCIAL ORGANIZATION OF HALICTINE BEES AND THE ECOLOGICAL SIGNIFICANCE OF SOCIAL FLEXIBILITY

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On-going studies show that social organization and life history attributes vary between populations of the neotropical sweat bee, Lasioglossum (Dialictus) umbripenne (Hymenoptera, Halictidae).

These populations are located along a north-south transect in eastern Panama, near the continental divide, and are separated by a distance of approximately 5 kilometers.

Colony size, social structure, and seasonal activity differ between these sites, but resource availability does not. Differences in seasonal activity may relate to local rainfall patterns.

Data from the Panamanian populations are compared with data from two conspecific populations in Costa Rica, which also differ in life history characteristics. Comparative data from temperate and tropical species show the great ecological success of halictid bees has been due, in part, to the flexible nature of their social behavior, which permits different phenotypic expression under different climatic conditions.

13-031

SIZE IN SOCIAL INSECTS--MULTIPLE COMPONENTS, MULTIPLE CAUSES

M. Kaspari

Body size is a key covariate to life history in unitary organisms. Social insect colonies, in contrast, are modular, making "body size" an intriguing concept. The mass of a social insect colony is (minimally) the product of *Size*--the mass of adults and larvae--and *Number*--the respective allotment to each. Size and Number are only weakly correlated across the ant species sampled, suggesting that they are largely under separate control.

I discuss the functional relevance of size and number in ant colonies, focusing mainly on allocation to the worker caste. I do this through a combination of literature review, field observations, and comparisons of the size makeup of ant assemblages from the Arctic Circle to Central America. I test the following hypotheses: 1) Size/Number is constrained by the architecture of the environment; 2) Size/Number is an optimal response to geographic variation in environmental productivity; and 3) Size/Number is an optimal response to survival during periods of resource shortage.

13-032

PATTERN AND PROCESS IN THE DEMOGRAPHY OF PAPERWASPS
(HYMENOPTERA: VESPIDAE, POLISTINAE)

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Demographic studies of social wasps have traditionally focused on the growth of single colonies. Stages of colony development have been named and described, and the population increase and decrease of individual colonies have been quantified within this descriptive framework. Studies of population demography (many nests) of social wasps are much less common, but that literature reveals that large numbers of foundress colonies never pass through the sequence of stages described in the colony-level studies. Natural history studies have additionally revealed that even for colonies that pass through the described stages, the potential reproductive activities of offspring are more numerous and varied than is typically reported. The population demography of paperwasps is thus more realistically modeled by a life cycle graph with multiple pathways than by the traditional model of sequential stages.

A life cycle graph has been developed and used as the conceptual framework for several seasons of preliminary experimental studies on the population demography of *Polistes metricus* in Missouri, U.S.A. Early season supplementation of honey led to larger colonies with more offspring but with a lower frequency of apparent worker behavior. Diminishment of larval saliva led to small colonies with low productivities and survivorship. Quantification of transitions in the life cycle graph remains to be undertaken, but the preliminary studies strongly suggest that typical colony ontogeny, and therefore also typical population demography, are shaped in part by the nutritional environment.

13-034

LATITUDINAL VARIATION OF SEASONAL DEVELOPMENT
STRATEGIES IN *MYRMICA* ANTS

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In *Myrmica* ants the duration of colony's annual cycle of development is controlled by an endogenous timer along with temperature and photoperiod. The seasonal development strategies of *Myrmica rubra* L. and *Myrmica ruginodis* Nyl. appeared to be quite different in Belgorod (50°NL) and St. Petersburg (60°NL) regions. The pupation of larvae originated from the eggs laid during the same summer (so-called rapid brood) starts early in July in Belgorod and only at the beginning of August in St. Petersburg when the weather is already rather cool there. That is why in Belgorod rapid brood is abundant whereas in St. Petersburg only a few rapid brood pupae appear. One could suggest that numerous rapid brood pupae in St. Petersburg do not develop because of direct influence of the inferior temperature in August. Natural spring colonies of both species were maintained in a laboratory at long (20 h) or short (12 h) days and 17, 20 or 25°C. Pupation of the rapid brood larvae ceased inevitably in all regimes but short days and low temperature significantly reduced the length of pupation period and the number of larvae pupated. St. Petersburg colonies appeared to differ from Belgorod ones by far shorter pupation period and much weaker rapid brood production at the same temperature and photoperiod. Thus, the dramatic difference between Belgorod and St. Petersburg populations in seasonal development strategies, which is a result of adaptation to really distinct climates, is not due to direct influence of temperature but is primarily determined by the innate endogenous mechanisms that regulate colony's development.

13-033

Social insect life history in different environments

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Bumble bees (*Bombus* spp.) often live in unpredictable environments. In addition, parasites are important selective factors for survival and reproductive success of colonies. We experimentally tested how unpredictable or low food availability affects productivity of colonies, the timing of reproductive events, offspring size and defence against parasites in lab colonies of *B. terrestris* exposed to simulated environments. When compared controls, low food availability, as expected, led to reduced colony growth with smaller workers, and fewer and smaller males. With unpredictable food supply, however, colony growth was increased with larger workers and heavier males. Furthermore, in all environments, the same level of parasite defence, as measured by the encapsulation response, was maintained. These results compare with conflicting field data and further experimental tests on the cost of parasite defence. Taken together, our conclusion is that colonies respond to unpredictability with increased production at a higher food acquisition level, and that other, usually more subtle critical needs, such as immune defence, may eventually become compromised.

13-035

ANNUAL DEVELOPMENTAL CYCLE STRATEGIES
IN TEMPERATE CLIMATE ANTS

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Temperate climate ants have heterodynamic seasonal development characterized by the presence of winter diapause. We distinguish two main annual cycle strategies in these social insects. The most widespread is the strategy of prolonged (extensive) development distinguished by a significant delaying of development of a large share of larvae (so-called slow brood) which continue to grow in autumn, hibernate in diapause and pupate during next summer. The appearance of larval diapause in evolution provided ants with several advantages: (1) workers can rear larvae from early spring to late autumn thus using the whole warm period of a year; (2) the development of larvae can be extended to two or even three summer seasons; (3) it is possible to adapt to the duration of the warm season by changing the quantity of so-called rapid brood (larvae that develop from egg to pupa without hibernation). The strategy of concentrated (intensive) development is characteristic to genera *Cataglyphis*, *Formica*, *Proformica*. The development of all brood stages is restricted to the warm season in these ants. Only queens have winter diapause; larvae finish their development during the summer, all new workers emerge from pupae and ants hibernate, therefore, without brood. This strategy can only be appropriate for northern ants in a combination with the very fast brood development allowing them to rear sexuals and new workers from the eggs during a short warm season. Among temperate ants *Formica* species have the shortest developmental times that are about twice lesser than, for example, in *Myrmica* and *Leptothorax*.

13-036

QUEEN ACOUSTIC BEHAVIOUR IN THE SICILIAN HONEY-BEE (*APIS MELLIFERA SICULA*)

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The acoustic behaviour of queens of *Apis m. sicula* was investigated during the swarming season of 1995 in Sicily and compared to the acoustic behaviour of queens of *Apis m. carnica*. Queen tooting has been shown to delay emergence from queen cells up to several days in *Apis m. carnica*. Since we could show that queens of *Apis m. sicula* do emerge from queen cells in the presence of free running queens, it was tested in this study, if tooting signals do occur at all in this honey-bee race, and if so, if they are different in any parameter from those of *Apis m. carnica* queens.

We found that tooting signals were produced by free running queens of the Sicilian honeybee and quaking signals by queens still in their queen cells. A comparison showed that these signals did not differ from the tooting and quaking signals of *Apis m. carnica* queens.

13-038

KING'S ROLE IN TERMITE SOCIETY: THE CASE OF *KALOTERMES FLAVICOLLIS* (ISOPTERA: KALOTERMITIDAE)

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Activities and social interactions of male and female replacement reproductives of *Kaloterмес flavicollis* were compared in an ethogram type investigation.

The study was conducted on colonies whose members, suitably representing all castes and developmental stages, were individually labelled. Data were collected using THE OBSERVER (Ver. 3.0, Noldus Information Technology, Wageningen), by scan-sampling all subjects of each colony from videorecorded images performed at preprogrammed intervals evenly arranged along the 24 hours, during a 16 days period.

Behavioural patterns of the king and the queen were similar in size, consisting in 13 elements, representing the behavioural categories of individual maintenance, feeding, communication and social interactions, but were extremely different in the frequency of some behaviours. The male reproductive appeared as the most active member of the colony. Its continuous shiftings within the nest and the abundance of vibratory movements, together with the high frequency of social contacts and peculiarity of grooming patterns, seem to confer to the king a very important function in termite society. These data suggest a sex-based behavioural specialization of the two reproductives in colony organization and equilibrium.

13-037

PHEROMONAL DOMINANCE SIGNALS IN PRIMITIVELY EUSOCIAL BEES

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In highly eusocial bees, queen pheromones regulate the dominance hierarchy and inhibit ovarian development in workers. Based on the results of behavioural experiments in primitively eusocial bumble bees and sweat bees, pheromonal dominance or recognition signals have been postulated, but have never been identified by chemical methods. In the primitively eusocial sweat bee *Lasioglossum malachurum* (Halictidae) and in the bumble bee *Bombus hypnorum* (Apidae) the behaviour of workers was observed in order to characterise them as foragers or guards in the sweat bee, and as dominant, subordinate or foragers in the bumble bee. Volatiles from queens and functional worker groups were chemically analysed and compared by statistical methods.

In *L. malachurum* queens, foragers and guards were different in their odour bouquets and in physiological traits. In *B. hypnorum*, dominant or subordinate workers and foragers could be characterised by physiological traits and by odour bouquets. In Dufour's gland-, head extracts, cuticular washings and headspace samples, queens, the worker groups classified by behavioural observations and age showed significant differences in both the total amounts and the relative proportions of volatiles. From all the worker groups, dominant workers showed the most similar volatile patterns in comparison to the queens. Behavioural tests with gland extracts and body surface washings indicated a communicative function as either recognition or dominance signals.

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13-039

A FEEDING STIMULATING SIGNAL IN THE LABIAL GLAND SECRETION OF THE TERMITE *RETICULITERMES SANTONENSIS* SJÖSTEDT (ISOPTERA: RHINOTERMITIDAE)J. Reinhard¹, M. Kaib²¹ Bundesanstalt für Materialforschung und -prüfung, Berlin, Germany² Lehrstuhl Tierphysiologie, Universität Bayreuth, Bayreuth, Germany

Chemical signals from secretions of different exocrine glands modulate a variety of behavioural patterns in termite societies. During food exploitation workers of the African termite *Schedorhinotermes lamanianus* Sjöstedt (Isoptera: Rhinotermitidae) release the secretion of their labial gland directly onto the food. The secretion carries a signal, which stimulates feeding and leads to aggregations of gnawing workers at feeding sites (Kaib & Ziesmann, 1992, Ins. Soc. 39, 373-384; Reinhard & Kaib, 1995, Physiol. Entomol. 20, 266-272).

The paired labial gland of the French termite *Reticulitermes santonensis* Sjöstedt (Isoptera: Rhinotermitidae) is located in the meso- and metathorax. In the head, the gland ducts join with the ducts of the nearby located water sacs. In a feeding bioassay, workers of *R. santonensis* had to choose between two semicircles of moist filterpaper as food. The termites significantly preferred one of the semicircles, if labial gland secretion was applied on it. That is, the labial gland secretion of *R. santonensis* carries a feeding stimulating signal as well. The content of the water sacs is not active in this context. The feeding stimulating signal proved to be extremely polar (soluble in water only), heat-resistant up to 100°C for at least 24h and thus to be very persistent. These chemical properties point to e.g. salts or saccharides as signal substances.

Apart from its own secretion, the labial gland secretions of 8 further termite species from 5 different families, including *S. lamanianus*, all proved to have this feeding stimulating effect on *R. santonensis*. This points towards a chemically similar or even identical principle in the labial gland secretion of termites.

13-040

SLAVE RAIDING BEHAVIOUR IN SOCIALLY PARASITIC *STRONGYLOGNATHUS* ANTS (HYMENOPTERA: FORMICIDAE)

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Slave raids were observed in five species of the Palearctic genus *Strongylognathus*, obligatory social parasites of *Tetramorium* host species, with a poorly known life history. In arena experiments we demonstrated that *S. caeciliae* FOREL 1897, *S. destefanii* EMERY 1915, *S. emeryi* MENOZZI 1921, *S. ceconii* EMERY 1916 and *S. silvestrii* MENOZZI 1936 are capable of conducting raids upon foreign *Tetramorium* nests, including host brood retrieval. Single raids differed considerably in various details, however, the following characteristic traits can be outlined. Scouting activity increases in the evening, and experiments were thus mainly performed during the night. Most impressive in *Strongylognathus* raids is group recruitment on pheromone trails. Fighting appears relatively obscure and inconspicuous, though the saber-shaped mandibles are used to penetrate into the central nervous system of defending *Tetramorium* via the mouthparts. During some fights even the strongly chitinized head capsule was eventually pierced. *Tetramorium* slaves join raiding parties and fighting, whereas brood transportation to the slave-makers' nest is carried out exclusively by *Strongylognathus* workers. Larvae, pupae and even adults are pillaged from a raided nest, and often all the conquered host colony was incorporated into the parasite society. Acceptance of foreign host colony queens after two raids may represent an artefact. Notwithstanding some minor deviations from typical slave-maker behaviour we thus found evidence for dulotic life habits in the *Strongylognathus* species studied.

13-042

TESTING KINSHIP THEORY WITH *ROPALIDIA MARGINATA*

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Ropalidia marginata is a primitively eusocial wasp in which new nests are initiated by one or a group of individuals. In the latter case, all but one individual take on the role of sterile workers. Per capita productivity does not increase significantly with number of foundresses and hence is not a potential explanation for the apparent altruism of the workers. Polyandry and serial polygyny significantly reduce intra-colony genetic relatedness so that kinship theory based on genetic asymmetries created by haplodiploidy is not a satisfactory explanation either. It is therefore of interest to examine the possible existence of intra-colony kin recognition that can potentially lead to nepotism. Young wasps, 0-8 days old, were readily accepted into alien conspecific nests. This may have partly to do with their lack of ovarian development but age *per se* was more important in influencing the probability of acceptance than ovarian development. In any case this provided a method for us to create genetically mixed colonies. In 12 such colonies studied, we could detect no evidence of kin-specific interactions, task specialization or nepotism. The alien wasps went on to become foragers or replacement queens with probabilities that were indistinguishable from those with which natal wasps did so. These results suggest that genetically unrelated wasps are as amenable to cooperative nest building and brood rearing as related wasps are. To test if there is any cost at all of living in genetically mixed colonies, we measured the productivities of pairs of wasps that were either nestmates or non nestmates and found no significant differences. Our results suggest that factors other than haplodiploidy based kin selection must be sought as explanations for the evolution of worker behaviour.

13-041

CHARACTERIZATION OF THE MANDIBULAR GLAND ORIFICE AND ITS CONTROL MECHANISM IN ANTS (HYMENOPTERA: FORMICIDAE)

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The mandibular gland in ants is generally known as the source of alarm pheromones. About the morphology and topographical organization of the control mechanism and the position of the glandular duct and its orifice, some inaccurate data were reported until now. This is due to the difficulties in making it accessible for morphological research because of the position of the mandible and the orifice underneath the heavily sclerotized fore margin of the head capsule. Dissected and critical point dried mandibles showed a narrow slit, starting near the mesal edge where the mandible articulates with the head capsule, then extending more anteriorly on the upper surface of the mandible and curving towards its inner margin. The part of the mandible where the mandibular orifice occurs, is known as the mandalus in literature. The aim of our investigation is to come to a comparative study of the control mechanism in species belonging to the different ant subfamilies. We observed a lot of variability of mandibular gland openings in different ant species. There is no direct relation between the size of the mandibular gland and that of the glandular orifice. When looking at the functioning of the control apparatus of the mandibular gland duct in ants, we see that the chitinous structure of the mandalus functions as a flexible plug, with a position that can be changed slightly by action of the musculus abductor mandibularis. Usually, when ants are alarmed, they open their mandibles towards the aggressor. This opening of the mandibles is assumed to be sufficient for the widening of the slit-like mandibular gland orifice. This assumption gains in importance when we look at semi-thin sections : no sphincter or other muscles could be detected in association with the mandibular gland duct

13-043

DYNAMIC OPTIMIZATION MODELING AS APPLIED TO PREDICTING LIFE HISTORIES IN THE SOCIAL INSECTS.

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Social insect colonies live in complex, always changing, environments. Clearly, one of the great advantages of sociality is the ability to exploit positive changes and to withstand negative changes. Previous models of colony behavior or ontogeny are static in considering the environment only in its expected, average state (if the environment is included at all!). However, with the advent of powerful personal computers, the effects of environmental variability can be studied and not ignored. The numerical techniques of dynamic optimization generate models that predict colony behavior in response to the simultaneous and changing factors of their own, internal needs and the external environment. Using such models we can ask questions such as:

1. What is a colony's optimal growth strategy in the face of changing levels of predation risk or competition?
2. What is the optimal allocation ratio between workers, soldiers and sexuals? And how sensitive should these ratios be to short term changes in the environmental conditions?
3. Do larvae serve as a cannibalizable caste under "normal" levels of food availability, or should they be eaten only under extreme stress? An example of such a model will be presented.

13-044

COLONY DEVELOPMENT IN THE BUMBLEBEE *BOMBUS TERRESTRIS* - A MINIMAL MODEL

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Bumblebees are annual eusocial insects. In early spring the overwintered queen starts a colony with the production of workers: the ergonomic phase. This phase ends when reproductives are raised: the reproductive phase. In the reproductive phase no more workers are produced. This is what is called a bang-bang strategy.

The more workers are present, the more reproductives can be raised (Pomeroy & Plowright 1982). But colonies that have a long ergonomic phase will start to reproduce late in the season. The risk of failure due to environmental hazards will be higher for late reproducing colonies (Müller & Schmid-Hempel 1992). Thus the colony faces a dilemma. Early reproduction decreases the number of reproductives, yet late reproduction increases the chance the colony will fail to reproduce at all.

Colonies of the bumblebee *Bombus terrestris* show a large variation in the number and sex ratio of reproductives they produce. Despite of several efforts to identify factors that influence the development of bumblebee colonies and thus the reproductive output, we still lack knowledge on the underlying functional mechanisms that direct reproduction in this bumblebee. Our model, which is an extension of the model of Macevicz and Oster (1976), is an attempt to fill this gap. Timing of the onset of the reproductive phase is optimal when the number of workers is sufficient to collect resources needed to raise the maximum number of reproductives at the end of colony lifetime.

Macevicz, S. & G. Oster. 1976. Modeling social insect populations II: Optimal reproductive strategies in annual eusocial insect colonies. *Behav. Ecol. Sociobiol.* 1: 265-282.

Müller, C.B. & P. Schmid-Hempel. 1992. Correlates of reproductive success among field colonies of *Bombus lucorum*: the importance of growth and parasites. *Ecol. Ent.* 17: 343-353.

Pomeroy, N. & R.C. Plowright. 1982. The relation between worker number and the production of males and queens in the bumblebee *Bombus perplexus*. *Can. J. Zool.* 60: 954-957.

13-046

NUMBER OF MATINGS IN THE GENUS *APIS* (HYMENOPTERA: APIDAE) REVEALED BY HYPER-VARIABLE MICROSATELLITES

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It has been postulated that multiple mating by social insect queens is adaptive because: 1) Genetically diverse colonies may be able to tolerate a wider range of environmental conditions, perhaps by increased polyethism or the potential for caste differentiation, or by increased tolerance to pathogens. 2) Multiple mating eliminates the possibility of a queen mating with a single drone carrying the same sex allele as herself. 3) Multiple mating can reduce conflict between workers and queens over the preferred sex ratio.

Understanding the evolution of multiple mating requires good estimates of the number of matings in a broad range of species. We have been using microsatellite markers to detect paternity and maternity of workers so that the number of males that mated with queens can be determined. Our results show that in the species so far examined, queens mated with > 6 to > 30 drones depending on the species. These results substantially increase previous estimates of the number of matings in the genus obtained from sperm counts.

13-045

Patterns of Genetic Diversity in the Polyandrous Leafcutter Ant *Atta colombica*

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Polyandry (ie. multiple mating by queens) in eusocial insects is considered an evolutionary puzzle. Polyandry is likely to be costly in terms of predation risk, time and energy expenditure for young queens and it reduces intracolony relatedness and hence inclusive fitness for reproductively altruistic workers. Two main hypotheses have been put forward to explain how polyandry might be favoured by natural selection. One hypothesis (Cole, 1983) suggests that multiple mating might be a queen strategy to obtain more sperm and thus be able to build up larger colonies and eventually produce more reproductive offspring. The other hypothesis focuses on potential benefits arising from the increase in genetic diversity brought about by polyandry (Hamilton, 1987).

We have carried out an analysis of queen sperm stores in the monogynous Panamanian leafcutter ant *Atta colombica*. The average ratio of queen to male sperm content was close to one. However, a DNA microsatellite genetic analysis of mother-offspring groups revealed *A. colombica* to be the most highly polyandrous ant yet reported (Fjerdingstad, Boomsma & Thorén, in prep.). We found no relationship at the individual queen level between genetic mating frequency and queen sperm content. Thus Cole's fecundity hypothesis for multiple mating can be rejected for *A. colombica*.

Hence, it seems that the evolutionary explanation for polyandry in *A. colombica* may be related to genetic diversity benefits. If more genetically diverse colonies have a higher survival fitness, eg. are more resistant towards parasites and pathogens attacking either the ants or their symbiotic fungus, then we might expect to find that the effective genetic diversity of mature colonies (which have succeeded in surviving for many years) would be higher than that of young, incipient colonies. We examine this using microsatellite markers.

13-047

DNA SEQUENCES REVEAL THAT SOCIALITY EVOLVED TWICE IN SOCIAL WASPS (HYMENOPTERA, VESPIDAE)

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For the evolution of sociality in wasps, the oriental Stenogastrinae are a group of considerable interest. They show broad diversity in social behavior. On the basis of morphological and behavioral data the Stenogastrinae are placed phylogenetically in the family Vespidae intermediate between the solitary Eumeninae and the social Polistinae and Vespinae. This placement of Stenogastrinae is not undisputed because of several morphological and behavioral characters that are very different from the other social Vespidae. However these autapomorphies are not suited to overthrow the existing phylogenetic placement.

Nucleotide sequences were obtained from the mitochondrial 16S ribosomal DNA, and the nuclear 28S ribosomal DNA region for two *Apis* species, and 12 social and three solitary wasp species of the family Vespidae. Solitary wasps of the family Ichneumonidae and Pteromalidae were used as outgroups. Parsimony and distance methods give strong evidence that the conventional phylogenetic position of Stenogastrinae is inappropriate. The solitary Eumeninae have to be placed as the sistergroup to Polistinae and Vespinae. This implies that sociality has independently evolved once in Stenogastrinae and a second time in a common ancestor of Polistinae and Vespinae. This is one time more than previously assumed.

13-048

NESTMATE RECOGNITION IN THE PRIMITIVE SOCIAL WASPS STENOGASTRINAE (HYMENOPTERA: VESPIDAE).

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The stenogastrine are generally considered the least socially evolved subfamily of wasps. Information collected during studies on the social biology of some species indicates that these wasps reject conspecific intruders from the nest. However, their nestmate recognition capacity has not yet been investigated.

We performed field experiments on nestmate recognition in *Parischonogaster jacobsoni* and two species of *Liostenogaster*, *L. flavolineata* and *L. vechti*. Conspecific hetero- and homocolonial females were presented to colonies of all the above mentioned species, observing the behaviour of the resident females. Experiments show that wasps are able to discriminate between nestmates and alien wasps. In particular *L. flavolineata* and *L. vechti*, which form large clusters of nests, reject also wasps coming from other colonies of the same nest cluster. These results suggest that the nestmate recognition of these wasps is very efficient, notwithstanding their poorly evolved sociality.

Behavioural tests on *L. flavolineata* show that, like other social insects, epicuticular lipids are the cues used for recognition.

Further experiments on *P. jacobsoni*, in which the wasps of a colony were faced with an alien nest containing immature brood, indicate a capacity for nest recognition. However the alien nests were always accepted and, in contrast with the massive destruction of alien immature brood observed in similar experiments in some polistine, brood was rarely destroyed in this species. In our opinion, the acceptance of alien nests and brood is not due to a lack of recognition capacity but is probably related to the great investment necessary for egg deposition and nest building in this subfamily.

13-049

NESTMATE RECOGNITION AND THE GENETICAL "GESTALT" OF *FORMICA POLYCTENA* COLONIES

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Social insects live in colonies and it seems to be common that they resist mixing with other colonies even of the same species. Individuals recognize those of other colonies through differences in odoural cues. Crozier and Dix (1979) developed a Gestalt model suggesting that workers respond to a colony odour which is produced by all colony members. Here we determine the genetic Gestalt of nests and look for its impact on nestmate recognition in the polygynous ant *Formica polyctena*.

Nestmate recognition was tested by measuring the aggression level between several individuals of different nests. The aggression was scored in arena tests on 3 level scale from 0 to 2. We found that one population (9 nests sampled in Berlin) had different levels of aggression (0.04 to 1.88) while the second one (7 nests sampled in Schwedt) showed low levels of aggression (0.07 to 0.52). 40 individuals of each nest were genetically typed as a DNA pool by multilocus fingerprinting, yielding the genetic composition of the whole nest (genetic Gestalt).

We found a strong negative correlation between the level of aggression and the similarity of DNA banding patterns between the genetic Gestalts ($p < 0.02$). Pairs with high aggression levels had fewer bands in common than pairs with low aggression. We suggest that nestmate recognition of ants in the species *Formica polyctena* is strongly influenced by the genetic composition of the whole nest as predicted by the Gestalt model.

Crozier and Dix (1979) Behav Ecol Sociobiol 4: 217-224

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13-050

THE FORMATION OF A "GESTALT" ODOR IN *CATAGLYPHIS NIGER*. DO QUEENS MATTER?

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The postpharyngeal gland (PPG) is an ant specific organ, present in all castes. Its secretion consists mostly of long chain methyl branched hydrocarbons which are congruent with those found on the cuticle. In workers the gland sequesters the hydrocarbons from both internal and external sources. By receiving and mixing hydrocarbons from multiple nestmates, the gland can be considered as a colonial reservoir of recognition cues that is shared by all the members of the society and thus responsible for creating the unified colony odor. We further evaluated the impact of the queen in this process in the colonies of *C. niger*. Chemical analyses of PPG secretions of queens and workers using GC/MS have shown that although the hydrocarbon content of the two castes is similar and queens are not much larger, they possess a significantly greater amount of hydrocarbons in their PPG. Interestingly, comparative studies on *de novo* hydrocarbon biosynthesis and their distribution using radiolabeled [¹⁴C] precursors have shown that although in both castes radiolabeled hydrocarbons were detected in the gland and on the cuticle, the total amount of hydrocarbons produced and their relative proportions in the gland were significantly higher in workers. Moreover, dyadic encounters between prelabeled queens and non labeled workers and vice versa revealed that although transfer occurs in all the cases, hydrocarbons were transferred significantly less from queens to workers than between workers.

Although, the discrepancy between the high hydrocarbon content of the gland and the low biosynthetic rate in the queen remains to be resolved, our results imply that in mature colonies of *C. niger*, the queen can not be considered as a main source of colony odor.

13-051

DO ANTS REARED IN A MIXED SPECIES GROUP RECOGNIZE ONLY THEIR NESTMATES' ODORS OR THE CORRESPONDING ALLOSPECIFIC ODORS IN GENERAL.

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Ants reared in a mixed species group (*Manica rubida* co-nesting with *Formica selysi*) constantly exchange recognition cues, resulting in a uniform group odor composed of substances produced by both species. To unravel whether the postpharyngeal gland is a source of recognition cues we tested the reaction of *M. rubida* to nestmates treated with glandular secretion from various sources.

Behavioral tests comprised two consecutive encounters between five ants and their color marked nestmate. The first (control) encounter was performed with an untreated ant, while in the second encounter the marked ant was treated with postpharyngeal gland exudate. Scoring was done using an aggression index obtained by direct observation, and an agitation index deduced from a frame by frame analysis of videotapes of the various tests. Tests were conducted with *M. rubida* originating from homospecific colonies or from a mixed species group. The postpharyngeal gland source was either from *M. rubida* or from *F. selysi*, as appropriate.

In tests using ants from homospecific colonies, applied with glandular secretions originating from an alien *M. rubida* ant or from a *F. selysi* belonging to a homospecific nest, the ants became very agitated and were aggressive towards their nestmate. In contrast, the application of exudates originating from a nestmate *M. rubida*, left the ants generally calm, although their rates of selfgrooming increased. Having established that the glandular secretion is a source of recognition cues, we repeated these experiments using *M. rubida* reared in a mixed species group. In general, the reaction of these ants was much milder and was graded depending on the source of the secretion. The highest aggression appeared towards ants applied with exudates of *F. selysi* from homospecific colonies. At the other extreme, the reaction towards ants applied with secretion belonging to *F. selysi* nestmates was not significantly different from the control.

The fact that worker *M. rubida* reared in mixed species group and exposed to a heterospecific colony odor are rendered more tolerant to ants bearing a similar bouquet, indicates their ability to generalize learned cues. Nevertheless the odor of nestmates or alien ants reared in similar mixed species groups seems to be the most familiar.

13-052

COLONIAL FUNGUS RECOGNITION OF THE ANT *ACROMYRMEX SUBTERRANEUS* (HYMENOPTERA FORMICIDAE)A.M.M. Viana¹, A. Lenoir^{1,2}

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We investigated the capacity of the fungus-growing ant *Acromyrmex subterraneus subterraneus*, Forel 1893, to recognize nestmate and non-nestmate brood. As brood is reared in the fungus which is a full part of the nest, we investigated also the specificity of the fungus. The different behavioural steps leading from discovery to transport into the nest, or reject in the dump of items encountered outside the nest were used to characterize the colonial recognition by workers. Tests were performed with larvae, pupae and fungus pellets. Nestmates (from the same colony: homocolonial), and non-nestmates, either heterocolonial (different colony) or heterospecific (other *Acromyrmex* species) items were used. Workers can easily discriminate between nestmates and non-nestmates. Homocolonial items were readily picked up and carried into the nest and placed in the fungus garden. Non-nestmate items (alien brood or fungus) were always rejected. The fungus is treated exactly like the brood, accepted or rejected according its origin. The colonial identity, a characteristic of eusocial insects, extends to the fungus. We discuss evolutionary mechanisms for this remarkable coevolution between an ant and a fungus.

13-054

STRUCTURE OF TERMITE MOUNDS - AN ADAPTATION TO AMBIENT TEMPERATURE?

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Since 1992, we are studying the influence of ambient temperature on the structure of *Macrotermes bellicosus* mounds in a West African Guinea Savannah. In the Comoé-National Park (Ivory Coast) the mound architecture differed greatly between the two adjacent habitats, shrub savannah and riparian forest, with highly structured mounds ('nids en cathédrale') in the former and less structured mounds in the latter ('nids en dôme'). By measuring the surfaces of the mounds directly with a new technique we found a quantitative correlation between ambient temperature and mound complexity. Experimental manipulations of the ambient temperature confirmed the causal relationship between these two variables. The dome shaped structure of mounds of *M. bellicosus* in the riparian forest is viewed as an adaption to this cooler habitat by reducing loss of internally generated heat to the environment. Evidence as to the adequacy of this hypothesis is given by data on the respective amount of energy required to heat mounds in both habitats.

13-053

STRATEGIES OF COLONY FOUNDING IN THE FACULTATIVE SLAVE-MAKING ANT *FORMICA SANGUINEA* LATR. (HYMENOPTERA, FORMICIDAE)

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Little is known about the mode of formation of new colonies in the facultative European slave-making ant *Formica sanguinea*. Nevertheless, the observation on colony founding by queens of this species is considered to be crucial for the understanding of the evolutionary origin of slavery (or dulosis) in ants.

To investigate this phenomenon, a series of laboratory experiments was conducted utilizing newly-mated females of *F. sanguinea* collected from polygynous pure and dulotic colonies. In particular, the following modes of colony foundation were studied: *i*) independent (one or more homocolonial females together); *ii*) in alliance with a female of host species; *iii*) for usurpation in an incipient or a mature host colony; *iv*) for pillage of host pupae; *v*) for adoption into an incipient or a mature queenright host colony; *vi*) for adoption into a incipient or a mature orphaned host colony.

Independent foundation was possible only when several females were kept together. Alliances followed by egg-depositions were obtained with females of two *Serviformica* species (*F. cunicularia*, *F. rufibarbis*). Usurpations and adoptions were more frequent in the incipient than in the mature host colonies. Mixed colonies were always obtained after the sack of the host pupae.

It seems likely that, besides homospecific adoption of queens followed by budding, *F. sanguinea* relies on temporary parasitism to start new colonies.

13-055

INVESTIGATION OF HONEYBEE BODY TEMPERATURE IN A WINTER CLUSTER BY INFRARED THERMOGRAPHY

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In temperate and cold climates overwintering honeybees regulate the core temperature of winter clusters in the range of 12-33.5°C (Fahrenheit et al. 1989, J Comp Physiol B 159: 551-560). In the present study we measured the *body* surface temperatures of bees (*Apis mellifera carnica*) at the periphery and along a cross-section through the cluster with minimum disturbance by means of thermography (Stabentheiner & Schmaranzer 1987, Thermology 2, 563-572). The colony was placed on 4 combs in a plexi glass hive which could be opened at the periphery and in the middle.

At ambient temperatures of 1-5.5°C, nearly all bees on the periphery (with only one exception) had thoracic temperatures (T_{th}) higher than 9°C: 9.5-12.5°C at the top, 10.5-19°C at the center and 8-15°C at the low end of the clusters. Head (T_{ca}) and abdominal (T_{ab}) temperatures ranged from 10-18°C and 6.5-15°C, respectively.

Along a vertical cross-section through a winter cluster with brood cells T_{th} ranged from 17-19°C at the top to 33-38°C at the core and 18-22°C at the low end. T_{ca} ranged from 18-19.5°C at the top to 31-36°C at the core and 15.5-20°C at the low end, and T_{ab} was 13-18°C at the top, 28-30.5°C at the core and 12-16.5°C at the low end.

Since in most individuals of the cluster surface the temperature of different body parts followed the rule $T_{ca} \geq T_{th} > T_{ab}$, these bees seem primarily to be heated passively by heat from the cluster's interior. Only in a few of the surface bees slight active heat production was indicated ($T_{ca} < T_{th} > T_{ab}$). By way of contrast, active heat production was clearly more frequent in bees inside the cluster, maximum heat production occurring in the center and lower half of the cluster.

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13-056

FEEDING FREQUENCY IN BUMBLE BEE LARVAE

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Caste in bumble bees may be, among others factors, influenced by food. Therefore, the feeding frequency of *Bombus terrestris* larvae (workers, males and queens) was studied by using video-recordings (4 hours a day), during the entire larval development.

The analysis of the tapes showed that in the beginning all larvae receive food at a very low frequency (less than 1 feeding in 4h). In the following days the frequency increases, reaching a peak one or 2 days before the end of the development (in average, 9 times for workers, 11 to males and 15 times to queens, approximately). Finally, all groups of larvae are fed less often just before pupation.

Worker larvae of the first brood have lower feeding frequency than the ones from the second and third broods. Male larvae receive food more often than workers, mainly in the last phase of their development. And queens have a much higher feeding frequency than workers and males, but only after the 10 th day of development.

Feedings do not occur regularly, i.e., the interval between two feedings may vary from a few seconds up to 3 hours or more. Obviously, the maximum intervals become smaller with the approximation of the peak of feedings.

Moreover, there is a large variation in the number of times different larvae of the same egg cell receive food, suggesting there are individual differences among the larvae and/or the feeders.

13-058

THE NASONOV PHEROMONE OF THE JAPANESE HONEYBEE, *Apis cerana japonica* Rad.

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Much of the knowledge on honeybee pheromones has been obtained from the European honeybee, *Apis mellifera* L. (Am). In this presentation, we demonstrate that the Japanese honeybee, *Apis cerana japonica* Rad. (Acj) utilizes different components of the Nasonov pheromone from those of Am.

GC and GC/MS analyses of the Nasonov gland extracts, and of the airborne volatiles of workers corroborated the occurrence of previously identified components in Am samples, but none of these compounds were found in Acj. By contrast, linalool and four types of linalool oxide (*cis* and *trans* of both furanoid and pyranoid) were identified in the Acj extracts. Chiral GC analysis revealed that the natural linalool oxide were identical to those prepared from (S)-(+)-linalool. In a laboratory bioassay, Acj workers placed in polyethylene bags were attracted (52-94%) to authentic linalool oxide (5-500ng, furanoid, racemate, 1:1 mixture of *cis* and *trans*). Neither Acj extract nor linalool oxide was attractive to Am workers. These results suggest that linalool oxide comprises the Nasonov pheromone of Acj.

Field bioassay using linalool and linalool oxide with natural absolute configurations and biological activities of other compounds in the extract will be discussed.

13-057

A PRELIMINARY WORK ON THE PUTATIVE GROUP SPECIFIC PHEROMONES IN ANTS

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Horstmann (1976) showed that red wood ant (*Formica polyctena*) foragers lay scent trails emanating from the rectal glands and that in protein or sugar-starved laboratory colonies, these scent trails caused directional recruitment to a protein or sugar source (Horstmann et al., 1982; Rosengren and Fortelius, 1987). Chemical constituents of these trail pheromones have not been identified yet but there is the possibility that they may also be specific for groups of foragers in the same colony, serving as directional cues for route fidelity.

To test the use of group specific pheromones in the red wood ant *Formica rufa*, worker responses to artificial trails prepared from gaster extracts of foragers taken from two branches of a field route were examined in the laboratory. The ability of foragers collected from one branch route to distinguish between scent trails to achieve route fidelity in darkness was tested.

The results showed that the distribution of these foragers between two artificial trails was random and provided no evidence for such group-specific pheromones.

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13-059

REPRODUCTIVE CONFLICTS IN COOPERATIVE ASSOCIATIONS OF FIRE ANT QUEENS (*SOLENOPSIS INVICTA*)

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In ants unrelated queens frequently associate to initiate a colony cooperatively. The joint reproductive effort of the cofoundresses increases growth and survival of the incipient colony. Yet, such associations are unstable. Soon after emergence of the first workers, queen-queen and queen-worker fights lead to the death or expulsion of all but one cofoundress. Because no sexual offspring are produced in incipient colonies the surviving queen monopolizes the entire future reproductive success of the colony. Two factors, the queens' relative fighting ability and their relative contribution to worker production (assuming that workers can recognize and selectively favour their mother) have been proposed to influence the survival prospects of individual queens within associations. The effect of these two factors were tested in the fire ant *Solenopsis invicta*. Initial size differences, a potential measure of the queens' fighting ability, affected the outcome of the conflicts, so that the initially heavier queen was more likely to survive. Differential weight loss by initially equal nestmates was also related to survival, with the queen losing more weight being more likely to die. The manipulation of the queens' relative contribution to the worker brood had no significant effect on the queens' survival probability, suggesting that workers are unable to favour their mother.

13-060

QUEEN-WORKER CONFLICTS AND SEX RATIO BIAS IN THE ANT *PHEIDOLE PALLIDULA*S. Aron¹, L. Keller², L. Passera¹, E. Campan¹¹Laboratoire d'Ethologie et Psychologie Animale, Université Paul-Sabatier, Toulouse, France - ²Institut de Zoologie et d'Ecologie Animale, University of Lausanne, Switzerland

Queen-worker conflict over the sex ratio of reproductive offspring in eusocial Hymenoptera provides a good model for testing the predictions of inclusive fitness theory and sex-ratio theory. To determine the extent to which workers and queens respectively control sex ratio in the ant *Pheidole pallidula*, we compared the primary sex ratio (at the egg stage) and the secondary sex ratio (reproductive pupae and adults) of colonies. There was a strong bimodal distribution of secondary sex ratios, with half of the colonies producing mostly females and other half mainly males. By contrast, there was no evidence of a bimodal distribution of primary sex ratios; all queens laid a same proportion of males eggs, decreasing from 0.40 in early spring to about 0.1 in summer. Moreover, the population sex-ratio investment was female-biased. These data are consistent with workers controlling the secondary sex ratio by selectively eliminating male brood in about half the colonies. We are currently testing whether the selective elimination of males is influenced by food availability and/or inter-colony variation in relatedness asymmetry, two factors that have been proposed to account for sex specialisation in social Hymenoptera.

13-062

PLASTICITY OF REPRODUCTIVE STRATEGIES OF THE FACULTATIVELY POLYGYNOUS ANT, *MYRMICA SULCINODIS*

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In polygynous social insects more than one queen is reproducing in a colony. This leads to a decrease in relatedness of workers to the sexual brood they are rearing, so that other cost benefit trade-offs than relatedness need to be considered to explain the evolution and stability of these eusocial systems. Environmental factors affecting survival and reproduction of queens are likely to play a role and if so, social and reproductive behaviour is expected to be plastic and to respond adaptively to changing environmental conditions.

Like the majority of its congeners, *Myrmica sulcinodis* NYL. has extensive variation in queen number, both between colonies within populations, and between populations. In this study we have examined the social and genetic structure of colonies from different habitats of the same population, using genetic information at nine polymorphic allozyme loci.

The study population inhabits two adjacent different habitat types. The main habitat is densely covered with heather. Here, *M. sulcinodis* occupies almost all suitable nest sites, which are rare patches of bare ground scattered across the heathland. The other marginal habitat is sparsely vegetated throughout, due to a recent fire. Here, most sites are unoccupied by ants. The genetic relatedness among nestmate workers, among worker brood, between workers and brood, between queens and workers, and between queens and brood was considerably higher for colonies in the marginal habitat than in the main habitat. However, the mean number of queens present in the nests did not differ between these habitat types.

From these observations it can be hypothesised that *M. sulcinodis* pursues two different reproductive strategies within the same breeding population. If free nest-sites exist, one or a few queens can establish new colonies on their own and reside as egg-layers for several seasons. Alternatively, if all nest-sites are already occupied, queens are adopted back into highly polygynous and polydomous (multi-nest) colonies that reproduce through budding. In these colonies queen turnover is fast and occurs even within the same season of egg-laying. Males are probably produced by workers whereas queens serve as the source of replacement queens and workers. The differences in social structure suggest that both queen and worker reproductive behaviour of *M. sulcinodis* are plastic and respond to the environmental constraints of changing population densities.

13-061

IS KIN CONFLICT EXPRESSED IN THE COLONY CYCLE OF THE BUMBLE BEE *BOMBUS TERRESTRIS*?

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The kin selection theory predicts that in the social Hymenoptera a conflict will arise between parent and offspring concerning the production of males. This study focuses on the production of males and the effects on the colony and discusses male parentage.

In *B. terrestris* the queen mates only once. After hibernation the solitary queen lays diploid eggs which produce the workers of the first and second brood. At some point in the third brood phase the queen switches to laying haploid eggs, from which males develop. These are nourished by the workers. The workers were expected to respond to the appearance of males by laying eggs themselves because they are related with the males of the queen for 0.25 only, but this does not occur. Worker behaviour does not change in response to male production by the queen either.

The workers start laying eggs after a eusocial phase of about a month. This transition is called the competition point. It is highly correlated with the moment at which queens, instead of workers, reared from diploid eggs. After the competition point only a few of the eggs, laid by workers and queen develop into adults. Most of the eggs are eaten. The queen plays an important role in the egg eating. The colony disintegrates and workers desert the colony.

By crossing two morphologically different sub-species of *B. terrestris* and taking measures to maintain brood rearing conditions after the competition point, it was possible to determine the share of the workers and the queen in male parentage after the competition point. It could be shown that, even after the competition point, the queen remains reproductively dominant. Matricide, a possible way in which workers can increase their fitness, occurred infrequently, and was not correlated with the competition point. The inclusive fitness of the workers is low not only because of a low direct fitness, most of the sexuals being derived from queen-laid eggs, but also because of the male-biased average sex ratio in the colonies, males being 4 times more frequent than queens.

13-063

SEX ALLOCATION OF *MYRMICA RUGINODIS* DEPENDS ON RELATEDNESS ASYMMETRY AND LOCAL RESOURCE COMPETITIONLaura Walin¹ & Perttu Seppä²¹Department of Ecology and Systematics & ²Department of Biosciences, University of Helsinki, FINLAND

The sex allocation of individual ant colonies may depend on the worker/queen ratio, different dispersal ability of the sexes or on the relative relatedness asymmetry of the colony. According to the inclusive fitness theory it is expected that the workers control the sex allocation of their colonies using these parameters when deciding on the optimal sex ratio. We studied the sex allocation of the red ant *Myrmica ruginodis*, which is known to have two morphologically and behaviourally distinct forms. Microgyna form is known to have more queens and nest formation often takes place by a simple budding of old colonies, whereas macrogyna colonies are usually monogynous and individual queens usually act as independent nest-founders.

A total of 35 whole colonies from a single population were excavated and sociogenetic organization and brood production were studied. Our results indicate that at the population level the sex allocation pattern is mostly affected by local resource competition due to poor dispersal of microgyna females. No indication of worker male production was found. When colonies were divided into two classes, mothered either by one queen or many queens, we could show that in the former class a significantly more female-biased sex ratio (as investment) was produced. Furthermore, the relatedness of nestmate workers was significantly higher in monogynous colonies.

13-064

PATTERNS OF REPRODUCTION IN SLAVE-MAKING ANTS

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We examined reproductive allocation in three slavemaking leptothoracine ants (*Leptothorax duloticus*, *Harpagoxenus canadensis*, and *Protomognathus americanus*). Because sex ratios in slavemaking ants have been proposed as a critical test case for the hypothesis that eusociality evolved via kin selection, we wished to test the hypothesis that slavemaker sex ratios reflect the queen's interests. We found little evidence to support the hypothesis, but instead our data showed evidence of conflict between the slavemaker queen and her workers. Furthermore, explicit comparisons of slavemaker sex ratios with their conspecific hosts showed that the slaves themselves may exert strong influences over sex allocation decisions in their parasites.

13-066

INSEMINATION REGULATES REPRODUCTION IN QUEENLESS COLONIES OF THE PONERINE ANT *GNAMPTOGENYS* SP. (PONERINAE: FORMICIDAE)

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14 queenless colonies of an arboreal *Gnamptogenys* sp. were completely collected and workers individually marked for observation. In these 1 to 14 inseminated reproductive workers (gamergates) were present, while most of the virgin workers laid trophic eggs of variable shape. We observed frequent reciprocal antennal boxing among the gamergates, but these or other aggressive interactions were rare among virgin workers and from gamergates to virgin workers. When virgin worker groups were created, this resulted in strong aggression among most individuals. This aggression led to a non-linear hierarchy with an alfa-worker at the top, but none of the workers ever started laying reproductive eggs. Dissections of workers of unmanipulated colonies as well as virgin worker groups confirmed that none of the virgin workers produced reproductive oocytes. Gamergate ovarian development was higher and only in these were yellow bodies found, thus indicating that insemination controls the type of eggs produced (trophic or reproductive). In 3 of the virgin worker groups that were observed for several weeks, dominant workers would eventually go out of the nest to perform sexual calling behaviour. When a single male was left in the arena of one such group, the alfa-worker got mated. This result suggests that dominance interactions regulate insemination. During the observations it became clear that virgin workers could recognize the gamergate from a short distance. However when two additional virgin worker groups were created by splitting one colony with a single mesh and the other with a double mesh, in both cases strong aggression followed. These results suggest that only in absence of physical contact aggression starts.

13-065

QUEEN DIMORPHISM IN THE NEOTROPICAL PONERINE ANT *ECTATOMMA RUIDUM* ROGER (HYMENOPTERA, FORMICIDAE)

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Known till now principally in Myrmicinae but also in Pseudomyrmecinae and Formicinae, this is the first report of the coexistence of two forms of reproductive females (microgynes and macrogynes) in a ponerine ant: *Ectatomma ruidum* Roger. The morphological study indicates the absence of intermediates between both forms. While similar in size and weight to the workers, the microgynes present a wider and longer thorax with functional wings and a wider head with three ocelli. In natural conditions as in laboratory, both forms are able to initiate a new colony using the semi-claustral mode of colony foundation typical for ponerine ant species. The relation "wing surface / body weight" is in favor of the microgynes suggesting a better dispersal capacity compared to the macrogynes. Nevertheless, with a number of ovarioles varying from 2 to 4 (median 3) vs. 3 to 12 (median 6), the fecundity of microgynes is less than macrogynes and the success rate of the colony foundation by microgynes is more limited as proved by the study of the colonies development from foundresses of both forms. The presence of microgynes seems to be associated with a reduced size of the colony, but there is not any evidence of a direct relationship with a variation of the population density or with the availability of food sources. Present in about 20% of the nests of *E. ruidum* collected in Mexico in the Rosario Izapa zone (Chiapas), the hypothetical biological function (i.e. an adapted strategy of reproduction) of the microgynes is discussed under a phylogenetic viewpoint in reference with what is known in myrmicines.

13-067

SOCIAL FACTORS AFFECTING ENDOCRINE MEDIATED REPRODUCTIVE DEVELOPMENT IN WORKERS OF *BOMBUS TERRESTRIS*.

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Colonies of *B.terrestris* are characterized by two major social phases. The eusocial phase in which the queen is the only reproductive female and a competition phase in which at least some of the workers reproduce, contributing to the male offspring's of the colony. A decrease in queen inhibition ability was proposed as an explanation for the onset of worker reproduction. In this study we compared the effect of queens from various social environments on worker reproduction and aggressive behavior.

Using a radio chemical assay (RCA) for JH biosynthesis by corpora allata (CA) *in vitro* that was optimized for *B. terrestris* we demonstrated that ovarian development are positively correlated with elevated JH biosynthesis. Young workers in queenright colonies had low JH biosynthesis rates, and their ovaries developed slowly. In contrast, some of the workers of the same age that were kept as small queenless groups had high JH biosynthesis, their ovaries developed fast and they were aggressive toward nestmates. These workers generally oviposited as early as six days old.

To verify whether queen inhibition declined with time we constructed groups that included callow workers with either virgin queens or queens of colonies before or during the competition phase. Queenless groups served as control in these experiments. There were no differences in the rates of JH biosynthesis by CA of young workers confined with mated queens from colonies before or after the onset of worker reproduction. Furthermore, threatening behaviors and overt aggression were similar and low in these groups. In contrast, some of the workers confined with virgin queens or in queenless groups demonstrated a significantly higher rate of JH biosynthesis threatening behaviors and overt aggression. When callow workers were introduced into intact colonies before or during the competition phase or into mature queenless colonies, they all demonstrated low rates of JH biosynthesis.

These results do not support the hypothesis that a decline in queens inhibition capacity leads to the competition phase.

13-068

THE ROLE OF INDIVIDUAL AND SOCIAL EXPERIENCE
IN EARLY DEVELOPMENT OF ANT BEHAVIOUR

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A great deal of the flexibility observed in the behaviour of individual ants can be attributed to learning and this raises the question as to how development of their behaviour is affected by early experience.

Species with different foraging systems were compared: *Formica polyctena* Foerst. and *F. sanguinea* Latr., with collective organization of foraging, and singly foraging *F. cunicularia glauca* Ruzs. The latter two species are said to be the pair of slave-makers and slaves.

Experiments in which the individual and the social experience of naive lab reared ants were manipulated, indicated that: 1. many of such activities as terrestrial locomotions, simple patterns of area-related search behaviour and of brood care behaviour as well as simple recruitment activity seem to be innate to ants; 2. when compare equally aged workers during the first hours and days of their lives, *cunicularia* individuals appear to be able to active operations much earlier than the other two species; 3. the main factor promoting changes of the ant behaviour is poor / rich environmental rearing conditions; 4. cross-social rearing conditions (young workers of *F. cunicularia* with older workers of *F. sanguinea* and vice versa) do not influence the behavioural parameters in slave-makers (*F. sanguinea*), but only in slaves, by modifying their shape according to slave-makers. 5. preliminary results show that a "peck order" system is innate to ants, while delicate social relations and the very complex forms of communications such as distant homing, seem to be determined mainly by learning processes.

13-069

DIVISION OF LABOR BETWEEN FORAGING
CASTES OF THE BLACK MARCHING TERMITE
HOSPITALITERMES MEDIOFLAVUS (ISOPTERA:
TERMITIDAE)

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Castes and division of labor in the black marching termite *Hospitalitermes* were examined in this study.

Instar-related polyethism between workers was discovered in *Hospitalitermes medioflavus*. The genus distributed over tropical rain forests in Southeast Asia is one of the open-air foraging termites. It makes long journey with continuous column to the foraging sites. There, workers make food balls cooperatively. Task allocation between workers is seen at the foraging sites; workers gnawing food materials and workers carrying food balls to the nest. In foraging workers, there were three peaks of size; major, medium and minor. Gnawers were composed of medium and minor workers, while carriers were of major and medium workers. And it became clear that the trimorphism was related to post-embryonic development, because there were no white larval instars corresponding to medium and major workers, and because molting workers were also found in the nest.

The temporal dynamics in foraging activities of the open-air foraging termite *H. medioflavus* was also investigated. Especially we focused on the dynamics of caste ratio during the activity. From the results differences of behavior between soldiers, major, medium and minor workers were identified. It is considered that soldiers play the roles of scout, defender and messenger and workers divide tasks to collect foods and carry them to the nest.

13-070

COLONY SOCIAL ORGANISATION, FORAGING COSTS AND
PONERINE ANT FORAGING STRATEGY.

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Two closely related ponerine ant species, *Ophthalmopone berthoudi* and *Megaponera foetens*, are both obligatory termite hunters, and overlap in their distribution, but use different foraging strategies; solitary hunting and group raiding respectively. This study investigated the constraints on these two foraging strategies by looking at the foraging costs of each strategy in relation to attributes of the ants' life histories.

The energy cost of an individual *O. berthoudi* forager was approximately 0.3% of the average foraging reward. In *M. foetens* the cost of a raiding party was approximately 30% of the foraging reward. Yet, *M. foetens* has raiding parties of approximately 300 workers while *O. berthoudi* has an average of 38 workers foraging during peak activity.

To evaluate an ant foraging strategy fully, the cost of producing sexuals has to be considered. *Ophthalmopone berthoudi* do not possess a queen but have laying workers (gamergates), only males need to be produced for the colony to reproduce by fission. However, in *M. foetens* both ergatoid queens and males are required for colony reproduction by fission. Thus the need for a greater net energy gain for production of sexuals may have contributed to the development of a more complex and efficient foraging strategy with a larger number of workers foraging.

13-071

EFFECT OF PERORALLY GIVEN BIOLOGICALLY ACTIVE SUBSTANCE OF ANIMAL ORIGIN ON MICROSPORIDIOSIS INFECTED HONEY BEES (*APIS MELLIFERA*)

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Biologically active substances (BASs) isolated from different animal systems and organs possess stimulative effect permitting to use them in medicine and veterinary. In this report we present some data concerning a BAS preparation, RBS, isolated from cattle embryo tissues and its effect on healthy and Microsporidia-infected (MI) honey bees (HB), both insects infected in laboratory and naturally infected having been used. In our laboratory experiments HBs were twice fed with 0,1 % RBS containing 50 % sucrose syrup (immediately after infection and 10 days post MI); in field experiments HBs were fed with RBS three times with 14 days intervals. In laboratory tests insects of the same age were infected in July-August (10,000 spores/HB); our field experiments were made with HB families with natural infection occurred in May-June. We found that RBS had no therapeutic effect in laboratory experiments. Furthermore, some MI stimulation took place accompanied by higher mortality of RBS-treated infected HBs comparing to untreated infected ones, the latter having smaller spores according to morphometric data. These facts are well correlated with a conception of obligatory parasitism postulating that factors favourable for a host organism are also favourable for its parasites, the disbalance developed in host-parasite system killing one of this system members.

The use of RBS in natural HB families with low MI frequency stimulated rather host than parasite organism, the treated families became stronger comparing to control ones. So it is necessary to be careful when treating parasites-carrying insects with BASs. Such preparations are to be used in apiculture only for HBs slightly infected or treated previously with antinosemic drugs.

13-073

AN ETHOLOGICAL CONTRIBUTION TOWARDS THE PHYLOGENETIC RELATIONSHIPS IN THE *Trigana* - *Tetragana* COMPLEX (APIDAE, MELIPONINAE).

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The cell provisioning and oviposition process (POP) of the stingless bees proceeds along a behavioral syndrome ruled by highly group-specific queen-workers interactions. The POP and related aspects involve 42 ethological characteristics of which 16 were used aiming a analysing the ethological phylogenetic relationships among representatives of the *Trigana* - *Tetragana* complex, namely the genera *Oxytrigana*, *Tetragana*, *Trigana* and *Cephalotrigana* of which only representative species was analysed. Such results (consistency index 0,88) may contribute to solve the polytomous approach used by Michener (1990) because they separate *Trigana*, *Tetragana* and *Cephalotrigana* (05 synapomorphies involved) and suggest *Oxytrigana* as the sister-group of the involved monophyletic clade. A comparison between such results and the positions held by Michener (1990) and Camargo & Pedro (1992 a,b) are provided.

13-072

VALIDATION OF THE DOUBLY LABELLED WATER TECHNIQUE FOR BUMBLEBEES *BOMBUS TERRESTRIS* (L.)

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In the doubly labelled water DLW technique, hydrogen and oxygen isotopes (deuterium or tritium and ¹⁸O, respectively) are added to an animal's body water. Isotope concentrations decrease exponentially with time through the natural 'washout' of CO₂ and water. The hydrogen isotope is lost as water only, and the oxygen isotope as both water and CO₂. Therefore, the apparent turnover rate of ¹⁸O is higher than that of deuterium (or tritium) and the difference between the two apparent turnover rates reflects the CO₂ production rate. The most outstanding feature of the DLW method is the ability to estimate the rates of body water turnover and the energy expenditure of free-living animals with minimal interference with their natural behaviour. This technique has liberated the study of vertebrate energetics from the artificial environment of the laboratory but has not yet been successfully validated for insects.

Here, I report its validation for the bumblebee *Bombus terrestris* (L.), using respirometry (RESP) from tethered roundabout flights. We injected small volumes (1 µl) of a mixture containing low concentrations of deuterium and ¹⁸O isotopes and withdrew 1-2 µl of haemolymph to determine initial ¹⁸O concentration. The injected isotopes were equilibrated with the body water pool after 10 min, and high material turnover allowed the analysis of final blood isotope concentrations after 5-7 h. On average (n=16), values measured using the DLW technique exceeded values using RESP by 3.1±9.9%, a difference which was statistically not significant at the 99% confidence level. The absolute error was 7.4±7.1% (mean ± S.D.). Isotope dilution spaces of both deuterium and ¹⁸O were almost identical with the body water pool.

The accuracy of DLW measurements allows its further use in field experiments. This could help to liberate the study of insect flight energetics from the restricting and unnatural environment of the laboratory. Energetic assumptions underlying many ideas in insect ecology could be tested directly. The small quantities of isotopes needed will help to restrict the costs of such studies, simultaneously allowing larger sample sizes.

13-074

LYSOZYME - AN IMMUNE FACTOR IN SECRETION OF SALIVARY GLANDS OF *APIS MELLIFERA* L

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We have found that apart from the previously known enzymes and pheromones each of three pairs of salivary glands of worker honeybees produces lysozyme (EC 3.2.1.17). This protein is able to lyse cell walls of bacteria. When entering the digestive tract of a bee, lysozyme imparts antibacterial properties to it, thus normalizing the microflora. Bees supply larval food, nectar, pollen, wax and propolis with lysozyme. It has been established that a high level of lysozyme secretion falls on the period of optimal functional activity of glands. Hypopharyngeal and mandibular glands produce the greatest quantity of lysozyme. The maximal activity of lysozyme in hypopharyngeal glands is revealed in nurse-bees on the 9th day of their life and also in field bees.

Since lysozyme is a factor of humoral immunity of insects, it is possible to conclude about the existence of a singular mechanism of the resistance formation in a honeybee colony.

The basic role in the production of lysozyme and saturation by this protein both of larval food and of food which is stored for wintering bees belongs to workers of summer generation.

13-075

USE OF STRIDULATION IN FORAGING LEAF-CUTTING ANTS: MECHANICAL SUPPORT DURING CUTTING OR SHORT-RANGE RECRUITMENT SIGNAL?

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Foraging leaf-cutting ant workers stridulate while cutting a leaf fragment. Two effects of stridulation have recently been identified: i) attraction of nestmates to the cutting site, employing substrate-borne stridulatory vibrations as short-range recruitment signals, and ii) mechanical facilitation of the cut, because the vibrating mandibles of a stridulating forager stiffen the tender leaf tissue like a vibrating knife. We asked whether foragers actually stridulate to support their cutting behavior, or whether the mechanical facilitation represents an epiphenomenon correlated with the use of stridulation as a short-range recruitment signal. To differentiate between both alternatives, workers of *Atta cephalotes* were presented with tender leaves of invariant physical traits, and their motivation to initiate recruitment was manipulated by varying the palatability of the leaves and the starvation of the colony. The lower the palatability of the harvested leaves, the lower the percentage of workers that stridulated while cutting, irrespective of the leaf's physical features. After intense feeding, no workers were observed to stridulate while cutting tender leaves, and the percentage of stridulating workers increased with deprivation-time. When confronted with familiar and unfamiliar leaves after intense feeding, while only 5 % of workers stridulated while cutting familiar leaves, as expected due to satiation effects, 60% of them stridulated while cutting unfamiliar leaves of similar physical traits. Results support the hypothesis that leaf-cutting ants workers stridulate while cutting leaves in order to recruit nestmates, and that the observed mechanical facilitation of stridulation during cutting represents a by-product of recruitment communication.

13-076

ENERGY EXPENDITURE DURING CUTTING AND LOAD-SIZE DETERMINATION IN LEAF-CUTTING ANTS

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Leaf-cutting ants cut vegetation into small fragments that they transport to the nest, where the material is processed by the ant colony's resident fungal garden. Here I report measurements on the energetic costs of leaf-cutting, and their relationship with the worker's decision about the size of the leaf fragment to be cut. Using open-flow respirometry, leaf-cutting energetics was measured in individual workers of *Atta sexdens*. The leaf-cutting metabolic rate (MR) was stable and dramatically above the post-cutting locomotion metabolic rate. Both leaf-cutting MR and post-cutting MR were strong functions of body mass. The aerobic scope (MR/StandardMR) of post-cutting locomotion was 7, and that of leaf-cutting was far larger, at 31. By comparison, the maximum sustainable metabolic rate in non-flying animals ranges from 8-12 times resting or standard metabolic rate, which increases to 20 - 100 for flying animals. Based on the observation that foragers cut smaller fragments, the harder the leaf tissue, similar measurements were performed by presenting ants with leaves of different toughness. Measurements of cutting speed, load-size selection and metabolic rate in individual foraging workers revealed that the total energy expenditure per fragment increased with increasing leaf toughness, and reached a maximum for a given leaf toughness which depended on ant body size. When workers harvested even harder leaves, they selected smaller leaf fragments, in such a way that the total energy expenditure per fragment remained similar for leaves of different toughness.

13-077

TEMPORAL POLYETHISM IN THE TERMITE *RETICULITERMES FUKIENENSIS* (ISOPTERA, RHINOTERMITIDAE)

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Measurements of head widths of workers and larvae from two colonies of *Reticulitermes fukienensis* revealed 5 size categories that could be readily distinguished. We termed the size categories WL1 to WL5. Larger individuals are on average older than smaller ones, since they have gone through more moults. WL1 and WL2 were typical larval morphology, with very white soft bodies, virtually unsclerotized mandibles, and clear gut contents. WL4 and WL5 were "worker" morphology with sclerotized mandibles (especially in WL5) and dark gut contents. WL3 was intermediate. Most behaviours monitored to date were carried out at highest frequency by WL5, the oldest individuals. Examples are given below:

- Scouting. When equal numbers of WL4 and WL5 individuals were placed under dark paper in a corner of the Petri dish, over 90% of the time WL5 ventured from under cover before WL4.
 - Tunneling. Tunnels made by isolated groups of WL5 individuals were over 10 times the length of those made by WL4.
 - Alarm behaviour. This behaviour was nearly 10 times more frequent in WL5 than in WL3 (WL4 was intermediate).
 - Burying corpses. Worker corpses were buried by WL5 individuals in 8 of the 10 replicates of WL5 but none of the ten replicates containing either WL3 or WL4.
- Current experiments are concentrating on larval rearing behaviours to determine whether younger individuals (e.g. WL3 and WL4) are involved more in these tasks or whether virtually all tasks are carried out mainly by older individuals.

13-078

DIVISION OF LABOUR WITHIN HONEYDEW COLLECTORS IN ANT SPECIES WITH DIFFERENT COLONY DESIGN

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Field observations and laboratory experiments have shown that adjustment of foraging efforts and task switching within a specialized category in an ant colony is connected with a level of social organization.

In the high social species *Formica polyctena* Foerst. each of an aphid colony is tended by a constant team including different groups. "Aphid ants" collect honeydew droplets and each member attend to about 3 individual aphids. "Watch ants" scare predators and alien ants away and they also protect aphids from drops in the rain. "Transporting ants" carry honeydew to the nest. "Discovering ants" search for new aphid colonies and sometimes they may take the place of other members.

In *Camponotus saxatilis* Ruzs. with more simple territorial structure, a lower level of individual specialization is revealed. One individual play a role of a "host" at the aphid colony and it combines the tasks of the "aphid ant" and the "watch ant" attending to about 15 individual aphids. There are also 2 - 3 "transporting ants". *Saxatilis* do not protect aphids from unfavourable influences.

Singly foraging *F. cunicularia glauca* Ruzs. may share aphid colonies with other species and each worker acts independently.

Preliminary results enable us to hypothesize that, at least in some ant species communities, when attending the same species of homopterans, ants species with more simple colony design may "steal" the honeydew from the homopterans without rendering any service in return and thus use "efforts" of high social species.

13-079

MORPHOLOGICAL CASTE DIFFERENCES IN *CHARTERGELLUS COMMUNIS* (HYMENOPTERA, VESPIDAE, EPIPONINI)**S. Mateus, F.B. Noll and R. Zucchi** (Financial support by Fapesp and CNPq)

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Neotropical swarm-founding polistine, in which polygyny is a rule, have remained less explored, mainly due to the difficulty for studying them continuously under both experimental and natural conditions. On the other hand, some sociological and other biological information on these wasps can be obtained, though partially, by analyzing a sample of wasps taken from a particular nest. This is especially true for the caste differentiation an related phenomena.

Caste differentiation analysis on *Chartergellus communis* evidenced a non-clear dimorphism among queens (inseminated egg-layers), workers (uninseminated with no ovary development) and intermediates (uninseminated with any ovary development). The measurements of 8 body parts and counts of hamulus number in a sample of 100 wasps showed that all characters of queens (n=19) were not significant larger than those of workers (n=54). Intermediates were significantly larger than workers in 6 caracteres and larger than queens in 7 caracteres (Kruskal-Wallis One Way Analysis of Variance on Ranks, $p < 0.05$). Canonical discriminant analysis showed intermediates with higher values in the first canonical variable (CAN_1) (-1.0 to 2.3) than workers (-2.7 to 1.8) and queens (-3.2 to 1.0). The most discriminant character was the basal width of the tergite II. The Mahalanobis's distances were 1.80 between queens and workers, 2.33 between queens and intermediates and 1.38 between workers and intermediates. These results evidence that caste differentiation in *C. communis* is not pronounced but intermediates are preferentially larger then the other kind of females. A closer analyses in the color patterns of frons, clypeus, mandibles and other related morphological body parts did not evidence inter caste differences.

13-081

SEXUAL INVESTMENT AND POLYANDRY IN THE LEAF-CUTTING ANT *ACROMYRMEX HEYERI* FOREL (HYMENOPTERA: FORMICIDAE)**E. Diehl-Fleig**

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Some of the most interesting problems in the evolutionary biology of formicids are related to the investment of colony resources in the reproductive brood, sexual rates of the colonies and populations, as well the colony social organisation. We aimed to analyse these questions in the leaf-cutting ant *Acromyrmex heyeri*. The males and gynes of three colonies at Torres (29°20'S; 49°43'W) and of three others at São Leopoldo (29°45'S; 51°08'W) were all numerically quantified. The energetic cost to produce each sex was estimated by the dry weight. As complementary data in other 22 colonies the sexual rate was qualitatively evaluated. The social organisation of each colony was obtained by means of five isoenzymic systems (MDH, α -GPDH, AMY-1, AMY-2 and AMY-4) analysed by horizontal electrophoresis.

Considering the number of adults of each sex, 67% of the colonies have a male bias production. However as the males' weight is lower than the females', only 33% of the colonies invested more resources in the male production. The low weight of the males can be reflecting not their small size only, but also a reduced quantity of sperm, that would be insufficient for the spermateca capacity. The isoenzymic analysis showed that 67% of the colonies are monogynous or polygynous, with the female mating with multiple males. The male bias production by the colonies reduces the energetic cost of the sexual production and can favour polyandry. The polyandry augments the genetic variability and as consequence favours a bigger adaptive plasticity. We did not find differences between the two sampled populations related to the sexual investment and social organisation of the colonies.

13-080

CASTE DIFFERENTIATION AND ITS RELATION TO THE COLONY CYCLE IN *POLYBIA (MYRAPETRA) PAULISTA* (HYMENOPTERA, VESPIDAE, EPIPONINI).**F. B. Noll and R. Zucchi** (Financial support by Fapesp and CNPq)

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Among the social insects, Epiponini tribe has challenged the students of behavioral social evolution because they generally present incipient caste differentiation and polygyny. These characteristics are sometimes interpreted as a complication to the evolution of social behavior via kin-selection theory (Hamilton 1964 a,b). However, recent studies (West-Eberhard, 1978) demonstrated that polygynous wasps colonies have their queen number diminished according to the cycle progression. Such facts seem to be related to population viscosity raising (Queller et al., 1990; Hughes et al., 1993).

Individuals from three colonies of *Polybia (Myrapetra) paulista* were morphometrically analysed in different phases of the colony cycle (foundation, worker production and sexuals production). Principal component analyses (PCA) indicated that larger queens are preferentially selected, what provokes caste differentiation increase according to colony age. In contrast, significant variation in worker morphology was not detected. In addition, generalized Mahalanobis distances (D^2) for each colony revealed the same trend in queen/worker differentiation previously observed (2.50 for foundation, 6.68 for worker production and 21.60 for sexuals production phases). Other fact is related to the appearing of intermediates (Richards and Richards, 1951) that were restricted only in the sexual production phase suggesting queen control decrease. These data imply that the colony cycle of epiponine reflects Polistinae phylogeny: Queens' morphology is initially undistinct from workers becoming statistically different later on. Such process recapitulates the evolutionary steps found in other related taxa.

13-082

MULTIVARIATE ANALYSIS APPLIED TO CASTE DIFFERENCES IN *SCHWARZIANA QUADRIPUNCTATA* (HYMENOPTERA, APIDAE, MELIPONINAE)**E. H. Nogueira-Ferreira, R. Zucchi & V. L. Imperatriz-Fonseca**

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Among the caste determination systems in stingless bees, namely interaction of genes and environment (Meliponini), and purely nutritional (Trigonini), the position of *Schwarziana* is outstanding because their gynes range from large (normal potential queens) until very small (miniature queens, same sized as true workers) (Imperatriz-Fonseca & Zucchi, 1995 and ref. therein). From 3 *Schwarziana* colonies, 42 queens (38 virgins, 3 physogastrics, one pupa from royal cell) and 39 workers were collected and measured from 11 morphological characteristics. Data were PCA (principal component analysis) treated using Systat programs, version 6.0. From the queens' sample 3 principal components were extracted which encompassed 79.6% of variance found. Graphically three distinct groups emerged, that are: 36 virgin gynes, 2 virgin gynes and 3 physogastric queens plus the analysed pupa. Among the analysed morphological characters, head width and maximum interorbital distance were the most discriminant ones. By including workers sample in the analysis, the 3 extracted components composed 76.6% of the variance found, and this time hind tibia length and malar area length are the characters that better express inter-caste separation. The analysis suggest: a- larger gynes have better chances of reaching the queen status and b- morphologically, such prospective queens are intermediate between workers and other kinds of gynes. Actually, it seems that caste determination in *Schwarziana* lies just in the border-land between the Meliponini system (abundant small gynes) and the Trigonini system (few, but larger gynes), irrespectively of the opinion held by Campos & Costa (1989).

13-083

ASPECTS OF SOCIAL BIOLOGY OF *EUGLOSSA TOWNSENDI* (HYMENOPTERA, APIDAE, EUGLOSSINI).

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Nests of *Euglossa townsendi* are founded by a single female that lays 4-13 eggs. After the last oviposition the foundress stays in the nest. When female daughters emerge, some leave the nest while others stay together with the mother in the natal nest and begin to reactivate it. The daughters (=foraging females) build, provision and oviposit their own cells. The foundress oviposits in cells provisioned and laid by foraging females and she dies before the second reactivation is started. The oviposition by the foundress is always preceded by oofagy. In five reactivation processes observed the number of foraging females varied from 1 to 4 and the number of cells oviposited by them from 3 to 10. After these females stop their ovipositions they may stay in the nest until the next reactivation. During the new reactivation processes those females perform oofagy and oviposition in cells laid by new foraging females. According to the number of those females, oviposition in a single cell occurs several times; the ovipositions are preceded by oofagy. Some new females may stay in the nest and perform only oofagy and ovipositions in cells laid by foraging females. The results obtained up to now suggest that unlike *E. cordata*, in *E. townsendi*, neither the presence of a single dominant female nor the aggressive interactions among the females in the nest occur.

13-085

TAXONOMY OF JAPANESE *RETICULITERMES* (ISOPTERA: RHINOTERMITIDAE) - MORPHOLOGICAL AND CHEMICAL APPROACH

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The genus *Reticulitermes* (Holmgren) is widely distributed in the northern temperate zone, and it occurs from Hokkaido to the Ryukyus in Japan.

In Japan, 3 species of *Reticulitermes* were recorded; *R. speratus* (Kolbe), *R. flaviceps amamianus* Morimoto and *R. miyatakei* Morimoto, and there is an undescribed species which is distributed around Yamaguchi area. *R. speratus* was subdivided into 5 subspecies based on the characters of soldiers and alates. Although, the morphological characters which were used to distinguish these species are continuous and Japanese *Reticulitermes* cannot be divided distinctly by these characters.

Recent studies on the cuticular hydrocarbons have suggested that components of them would be a variable taxonomic characters.

In this study, I identify the cuticular hydrocarbons of Japanese *Reticulitermes* and make comparison of these components, and re-evaluate the morphological characters. From this results, I suggest that there are 6 species of *Reticulitermes* in Japan. In addition, I discuss about these speciation.

13-084

FORAGING ACTIVITY OF THE LEAF-CUTTING ANT *Atta sexdens rubropilosa* Forel (FORMICIDAE: ATTINI) TO TEMPERATURE, HUMIDITY, AND ILLUMINATION

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This study describes the diurnal and nocturnal foraging patterns of the leaf-cutting ant *Atta sexdens rubropilosa* Forel in response to temperature, humidity, and illumination in a forest of eucalyptus (*Eucalyptus* spp.) located in Rio de Janeiro State, Brazil. There was no difference in average mass between diurnal and nocturnal foragers. However, nighttime foragers carried significantly lighter burdens than daytime foragers. It is suggested that daytime foragers carry heavier plant fragments to compensate for their low density on foraging trails when compared to nighttime foragers. Load, defined as (ant mass + fragment mass)/ant mass and fragment mass were significantly and negatively correlated with ant mass, although r^2 -values were low. The roles of temperature, humidity, and illumination on foraging activity of *Atta sexdens rubropilosa* are discussed.

13-086

TERMITES BIODIVERSITY ON DATE PALMS IN KHUZESTAN, IRAN AND PRELIMINARY STUDIES ON COLONY FOUNDATION IN ANACANTHOTERMES VAGANS (H.) IN LABORATORY CONDITIONS (ISOPTERA: HODOTERMITIDAE)

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Department of Entomology, College of Agriculture, Shahid-Chamran University Ahwaz, Iran. During 1992-1994 a faunistic investigation was carried out on termites (Isoptera) infesting date palms in Khuzestan (southwestern province of Iran). In this study a total of 5 species from 2 families were collected and identified in the province. Three species namely *Microcerotermes diversus* (S.), *M. buettikeri* (Ch.&B.) and *Amitermes vilis* (H.) were mostly collected and associated with date palm trees in the area. Whereas two other species namely *M. gabrielis* (W.) and *Anacanthotermes vagans* (H.) were generally found in the soil and in pasture lands respectively. They were not directly associated with date palm trees. Observations on colony foundation in *A. vagans* (H.) in lab. conditions revealed that the queen was able to lay first series of eggs about 7 days after colony foundation. Mean number of eggs which were laid during 9 and 28 days were 24 and 32 respectively. The time required for eclosion of the eggs and appearance of the larvae was observed to be 35 days. After 7 weeks from oviposition, the first workers of *A. vagans* (H.) were appeared in the colony. The rate of egg laying was declined and a second phase of oviposition was then initiated about 15 to 20 days after last eggs were hatched.

13-087

POPULATION DYNAMICS AND NUTRITIONAL BEHAVIOUR OF *MICROCEROTERMES DIVERSUS* (S.) IN AHWAZ, IRAN (ISOPTERA: TERMITIDAE)

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Some aspects of biology of *M. diversus* (S.) including relationship of foraging activity to seasonal cycle of nymph production, their development into alates, density of larval stages and temperature were studied.

An experiment was carried out in a piece of termite infested land burying paper rolls as source of food. Weekly collections were made of workers and nymphal instars 3-5 from buried paper rolls.

Also 10 more colonies were chosen in different localities in Ahwaz. They were inspected at 10 days intervals for a complete year, and all of the termites including larval stages, unpigmented workers and nymphal instars 3-5 were counted. Preliminary studies were also made on colony foundation in *M. diversus* (S.) in lab. conditions to help understand some aspects of nutritional behaviour of the species in the field.

It was observed that the first eggs were laid in breeding containers 8 to 9 days after colony foundation.

The times required for eclosion of eggs and appearance of workers were between 18 to 26 and 36 to 37 days respectively.

The result of investigation revealed that high activity of colony for production of alates and workers (presence of nymphs and larvae in large numbers in samples) from April through mid-November (indicating population increase in colony) correlated positively with foraging activity of the termite.

13-089

THE DRUMMING BEHAVIOUR OF THE CARPENTER ANT *CAMPONOTUS LIGNIPERDA* - INDIVIDUAL REACTIVITY AND GROUP BEHAVIOUR

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Old colonies of the carpenter ant *Camponotus ligniperda* usually live in nests inside of pinetrees build by gnawing out the soft wood of the annual rings and constructing thin concentric cylinders of wooden plates. When receiving any disturbing stimuli (CO_2 -Puff, ground vibration) they display a typical behavior (running in circles, mandibel gapping, biting attacks) and produce vibrational signals by alternately drumming with mandibel and gaster on the ground. This signal is perceived by nestmates up to a distance of 15cm and is discussed as a part of a "modulatory communication system" (Fuchs 1976).

We examined the meaning of individual behavioural variability for the performance of group-demands in an experimental setup simulating natural conditions. In individual and group-tests slowly walking workers receiving a simulated drumming signal show a startling response (standing still with stretched antenna) lasting several seconds. There is evidence that

1. the individual quality and duration of the startling response is a individual stable trait.
2. the response threshold for a second stimuli (different levels of CO_2) following the perception of the vibrational signal is modulated.
3. the perception of and the reaction to a drumming signal is influenced by species-specific chemical signals.

The drumming behaviour is discussed as a modulatory communication signal, fine tuned to physical features of a wooden nests and as a part of a elaborate multi-component alarming system.

Fuchs, S.: The Response to vibrations of the substrate and reactions to the specific drumming in colonies of Carpenter Ants (*Camponotus*, Formicidae, Hymenoptera). *Behav. Ecol. Sociobiol.* 1, 155-184 (1976)

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13-088

SWARMING AND FORAGING ACTIVITY OF SUBTERRANEAN TERMITE, *PSAMOTERMES HYPOSTOMA*, DESNEUX (RHINOTERMITIDAE) IN THE NEW VALLEY, EGYPT

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Flight and surface activity of *Psamotermes hypostoma* Desn. was investigated in the New Valley, Egypt. This termite species has two main periods of swarming. The first period lasted from February to May and the other extended from August to November. No swarming was recorded during hot (temp. $> 35^\circ\text{C}$) or cold ($< 10^\circ\text{C}$) months. Temperature, relative humidity, wind speed and certain biological factors were found to be the most important factors affecting flight activity of *P. hypostoma*.

Concerning the surface activity of this species, two peaks of foraging have been recorded. One was observed during March-April and the other during July-August. The lowest foraging periods were recorded during December-January and May-June. Low temperature during winter months was the main factors of reducing the foraging activity, whereas high temperature and the swarming activity were the main factors of reducing foraging during summer months.

13-090

TERRITORY, FORAGING ECOLOGY, LIFE HISTORY, TRANSPORT SYSTEM AND LONG DISTANCE RECRUITMENT OF THE GIANT ANT *CAMPONOTUS GIGAS* IN THE RAINFOREST OF SABAH, BORNEO

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The giant forest ant *Camponotus gigas* is one of the largest ant species in the world, with the spectacular major workers reaching occasionally 30 mm in length. This common inhabitant of the dipterocarp forest in Borneo, Sumatra and Peninsular Malaysia is mostly active by night, living in underground nests between the roots of overmature trees. At dusk hundreds of ants are climbing up the trunks, to forage in the forest canopy. In the 44 000 m^2 of our research plot, we observed 4 polydomous colonies of *C. gigas*. Each of the colonies had up to 14 nests, one of them covering a territory of 8000 m^2 . Not more than 8000 foragers were found in the colony. The foraging behavior of this ant species is highly organized. Specialized subcastes are working as hunters, transport workers or herdsmen. Its logistic functions according to the central-place foraging theory. 90% of its diet consists of honey dew from the homopteran *Bythopsyrna circulata* (Flatidae) and other trophobionts including true bugs (Coreidae) and Fulgoridae. As a source of nitrogen it uses dead arthropods and birds droppings. According to our observations at one colony, mating flight takes place only twice a year with intervals of approx. 188 days. We observed 4 flights comprising between 40 to 200 reproductives, mostly males. The territorial behavior of this ant is highly ritualized, with small groups of Majors (up to 5) involved in permanent fighting („boxing“). Interspecific borders to other *Camponotus* species are guarded by sentinels. To observe the cascade like long distance recruitment we constructed about 400 m artificial trail, mostly made of bamboo. To supervise this routes we used a data logger connected to light barriers.

13-091

EXPERIMENTAL REMOVAL OF MAIN PREY FORAGERS IN A PAPER WASP, *POLISTES JADWIGAE* (HYMENOPTERA: VESPIDAE)

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In post-emergence colonies of a paper wasp, *Polistes jadwigae*, main prey foragers were experimentally removed in order to learn how other wasps would respond to the reduction of prey brought into colonies, and to possible increase in larval hunger level resulting from it. The experiment was made with four colonies containing the queen and 8 to 12 workers, and 2 orphan colonies containing 11 or 12 workers. The colonies were each observed for 12 hours on three consecutive days: on the first and the third day, wasps were observed for behavior under undisturbed conditions; on the second day, 2 to 4 workers that most frequently came back to the nests with prey loads on the first day were removed early in the morning and behavior of other wasps was observed (the removed workers were released after the observation).

Removal of main foragers did not affect foraging activity of other wasps: number of prey loads brought into the nests by non-removed workers did not change between the first and the second day. Likewise, their activity level (proportion of time spent in acts other than grooming, fanning, and standing (=motionless)) or the number of their foraging trips did not change. In addition, in the 4 queenright colonies, queen activity level did not increase after the worker removal. These results contrast with those of Gamboa et al. (1990; *Ethology*, 85: 335-343), who suggest that queens are sensitive to brood needs, and that they behaviorally regulate worker foraging to match brood needs by increasing their level of activity in *Polistes fuscatus*. Regulation of worker foraging, if any, seems to be accomplished by agent(s) other than queen control in *Polistes jadwigae*.

13-093

STUDY ON AN AGGREGATED NEST OF THE BEE *ANDRENA AGILISSIMA* (HYMENOPTERA: ANDRENIDAE)

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A nest site of *Andrena agilissima* was studied at the beginning of May of the years 1993/95 at Isola d'Elba (Toscana). This species shows a strong trend towards nesting in aggregation, with a nest density of more than 100/m². During this research several hundred bees were marked. The observation of their behaviour gave the following results:

1. Foraging activity shows two peaks at mid morning and early afternoon, with a reduction of the foraging trips around noon;
2. Every nest is inhabited by several bees. Females move from one nest to another using underground tunnels;
3. Data about any level of sociality were not available. Yet threats or aggressions of any kind were not observed at all among interacting females, nor differences in the development of ovarians.
4. Parasitism by the bee fly *Bombylius fimbriatus* (Diptera, Bombyliidae) was detected.

13-092

DIFFERENTIATION OF THE STINGING MECHANISMS IN QUEEN AND WORKER HONEYBEES, *APIS MELLIFERA*: FUNCTIONAL AND STRUCTURAL ANALYSES

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Female honeybee differentiates into worker or queen. Virgin queen stings her reproductive rivals (sister queens) and workers do intruding (non-nestmates) workers and animals which disturb their nest. We compared the caste-specific differences in the stinging mechanism and venom function.

1) Aged workers showed active stinging response to mechanical stimuli but young ones within 24 h after emergence showed no response. Queens responded weaker than aged workers. However, decapitated bees showed strong response even in day 0, revealing the implication of inhibition from head, probably by brain. The inhibition can be cancelled by JH treatment.

2) Rhythmic movements of muscular systems (M197, M198 and M199) of the sting apparatus were recorded with electromyography (EMG): While mechanical stimuli caused similar rhythmic movements in both castes, sensory inputs from proprioceptors distributing over sting surface and/or the nerve severance between 6th and terminal abdominal ganglion (simulating sting autotomy) caused the movements only in workers. Quick and repetitive injections of the limited amount of venom by queen sting seemed to be under central control.

3) Ether-washed queen or isolated queen abdomen was enough to release the stinging response of assaultive queens. Neither 9ODA nor 9HDA released the behavior. Instead, the cuticular surface structure was found to play critical role in the queen-queen recognition. The cuticular surface dimorphism was found in *A. cerana* and *A. florea* also.

4) In comparison to worker venom, queen venom was 3 times more in amount and 50% higher in LC₅₀ when injected to worker bees.

13-094

FUNGUS-GROWING TERMITE IN JAPAN

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Seventeen species of termite were distributed in Japan. The fungus-growing termites belong to genera: *Hypoterme*s, *Macrotermes*, *Microtermes*, *Odontotermes*, *Canthotermes* and *Prototermes*. Only one species *Odontotermes formosanus* Shiraki was known in Japan. This termite makes fungus comb under ground. They inoculate mycelium to the nest. The mycelium spread in the nest and forms nodules. The mycelium and nodules were used as food for termite.

In rainy season (from the last seven days of May to July), the pseudorrhizae of the fungus developed on the comb. They break through the soil and forms carpophore on the ground. This mushroom was known *Termitomyces clypeatus* and *Termitomyces eurhizus*.

In order to cultivate this mushroom, the analysis of comb was done.

The combs were collected from Okinawa, Ishigaki and Iriomote island. The comb have the appearance of sponges, brains and corals. The comb floor is flat or little arched, the ceiling is domed. The diameter and weight of collected combs were about 25cm, 250g. The combs took out under ground was soft and brittle, but dried comb were very hard.

13-095

MORPHOLOGICAL DIFFERENCES AMONG FEMALES OF THE EUSOCIAL ALLODAPINE BEE, *EXONEURELLA TRIDENTATA* (HYMENOPTERA: APIDAE).

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Exoneurella tridentata is the only allodapine bee in which females fall into one of two groups based on size and morphology. Female pupal weights are strongly bimodal with, on average, the larger females (majors) being twice the weight of the smaller females (minors). Also, majors have reduced scopae on the hind legs and enlarged metasomas. Brood are not raised in cells but in a communal undivided chamber, yet by the pupal stage females fall into two distinct weight groups. This suggests that there is a developmental switch from the minor morphology, which is typical of allodapines, to the unusual major morphology.

The majority of *E. tridentata* colonies are multifemale. Colony size ranges up to 37 females per nest, which for allodapines is very large. Minors are more numerous than majors but most nests contain at least one major. When nests are opened the first bee encountered is frequently a major. In other bees morphological differences are associated with foraging or egg laying, rather than with guarding.

E. tridentata does not excavate its own nest, unlike most other allodapine bees, but utilises the burrows created by beetle larvae in the dead wood of long lived semi-arid trees. Other invertebrates also use beetle burrows which suggests that burrows may be a limiting resource. In *E. tridentata* the major morph may have evolved as an adaptation to colony defence with reproductive division of labour evolving later and resulting in eusocial colonies.

13-097

CLIMATE-MEDIATED SOCIALITY IN ALLODAPINE BEES.

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Opportunities for sib-rearing and the exploitation of high levels of relatedness among haplodiploid sisters arise only if some brood reach imaginal stages when younger dependent brood are still in the nest. In most insects, this requires that adults are long lived (> one year) or that multiple broods are produced in a single year. Studies of Australian allodapine bees have provided some evidence of sib-rearing in warmer, northern habitats, but this appears to be precluded in cooler climates where development rates may be lower. This suggests climate may be an important constraint in the development of eusociality. The current study investigated life-cycles and social behaviour in a number of allodapine bees along the east coast of Australia, where habitats ranged from sub-tropical in the north to temperate in the south. Voltinism and opportunities for sib-rearing varied with latitude such that eusociality was common in northern habitats but not in cooler, southern regions. Similar variation has been observed for other bcc taxa in other continents and demonstrates the importance of large-scale physical factors on social behaviour, and raises considerations for their impact on the evolution of sociality.

13-096

THE ECOLOGY AND SOCIAL BEHAVIOR OF AN ARID-ZONE ALLODAPINE BEE, *EXONEURELLA EREMOPHILA* (HYMENOPTERA: APIDAE: ALLODAPINI)

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Social variation among allodapine bee species provide unrivalled material for comparative studies of social evolution. The endemic Australian genus *Exoneurella* comprises four species which range from highly eusocial to subsocial. *E. eremophila* is a distal species in the *Exoneurella* clade. It is unusual in that it lives in a strongly xeric environment and multifemale colonies are very rare during brood rearing periods. Both these traits appear to be derived; nearly all other Australian allodapines are predominantly social and have a southern temperate distribution, probably reflecting a Gondwanaland origin. *E. eremophila* nests in the dead stalks of several herbaceous plants and grasses; these substrates deteriorate rapidly, limiting the potential for multifemale colonies to develop via re-use of natal nests by subsequent generation. Multifemale colonies occur primarily as "pre-reproductive assemblages", although some multifemale colonies may develop in sturdy nesting substrates. *E. eremophila* provides an opportunity to study behavior in a species where solitary nesting is a derived trait and where sociality is largely precluded by physical constraints.

13-098

SOCIAL EVOLUTION IN THE ALLODAPINE BEE GENUS *EXONEURELLA* (HYMENOPTERA: APIDAE): INSIGHTS FROM PHYLOGENY.

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Interspecific variation in sociality in allodapine bees provides an opportunity to study patterns and processes of social evolution. Of the four endemic Australian groups, *Inquilina* is socially parasitic, *Exoneura s.s.* and *Brevineura* display well-developed forms of social organization, and members of the group *Exoneurella* span the range from largely solitary to highly eusocial. *Exoneurella eremophila* and *E. setosa* are largely solitary, *E. lawsoni* is weakly social, and *E. tridentata* is highly eusocial with morphologically differentiated castes. Phylogenetic reconstruction indicates that *E. tridentata* is basal to this group and *E. eremophila* and *E. setosa* are distal. The characteristic of predominantly solitary nesting in the distal members of this group appears to represent a reversion from more social life-cycles, rather than being an ancestral trait. This reversion coincides with a radiation into xeric and saline environments where nesting substrates deteriorate rapidly, limiting colony growth over time. The potential for nesting substrates to limit expression of social behaviour has also been demonstrated for some species of *Exoneura s.s.*. It has previously been assumed that the range in sociality of Australian allodapines provides an opportunity to study early steps into sociality; our findings suggest that the opposite may be closer to the true situation.

13-099

SOCIAL AND SOLITARY BEHAVIOUR IN THE ALLODAPINE BEE *EXONEURELLA SETOSA*. (HYMENOPTERA: APIDAE)

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The allodapine bee group *Exoneurella* provides unequalled opportunities for comparative approaches to social evolution. *Exoneurella setosa* is a phylogenetically distal member of this group and is largely solitary. Solitary nesting appears to be a derived rather than ancestral character. Solitary behaviour is linked to occupation of coastal saline environments where nesting substrates rarely persist long enough to permit colony growth over more than one generation. Other factors associated with solitary nesting include a high level of physical disturbance affecting nest sites and low levels of ant predation compared to social allodapine bee species. Evidence for relictual caste differentiation in the small percentage of multifemale colonies that occur is presented and discussed with respect to phylogeny. Despite the rarity of social colonies during brood rearing, pre-reproductive assemblages during winter are common and the selective pressures maintaining these are discussed with respect to sex allocation patterns and Local Fitness Enhancement.

13-100

INTRA-COLONY AGGRESSION AND HIGHLY EUSOCIAL BEHAVIOUR IN AN ALLODAPINE BEE, *EXONEURELLA TRIDENTATA*.

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Examination of a native Australian allodapine bee, *Exoneurella tridentata* provides an opportunity for understanding processes in the evolution of morphological castes in social insects. *Exoneurella tridentata* has the largest known colony sizes during brood rearing periods for any allodapine bee. The species is also unique in that it displays morphological caste differentiation which is more extreme than in other social bee species. A behavioural study of *E. tridentata* revealed that unlike other allodapines, a high level of aggressive interaction (in the form of biting) was displayed between individuals within a nest. Agonistic behaviours have not been recorded for any other *Exoneura* species, and only rarely in other allodapines. Surprisingly, analyses of behavioural repertoires did not show that morphological caste was clearly linked to any particular behaviour(s), and that within nests only weak behavioural specialization occurred. The behaviour of *E. tridentata* within nests is very different to that observed for other highly eusocial species; it appears that this species is facultatively highly eusocial. It is suggested that morphological differentiation in this species probably arose from selective factors associated with nest defence, and reproductive specialization arose in the majors as a secondary function for this morph. These morphological castes may be the evolutionary outcome of a dichotomy in selective pressures - selection for guarding ability, and selection for more general tasks such as foraging. Selection for a morph which is effective at guarding may have lead to a morph which is also able to exert reproductive dominance.

13-101

OPPORTUNITIES FOR DISPERSAL AND SOCIALITY IN THE ALLODAPINE BEE, *EXONEURA BICOLOR*: COOPERATIVE NESTING IS NOT MAKING THE BEST OF A BAD SITUATION.

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Several factors thought to be important for the evolution of cooperative breeding in vertebrates have received little attention in facultatively social insects. One of these, Selander's (1964) 'habitat saturation hypothesis', predicts that colony sizes will be greater in breeding units where dispersal opportunities are limited, suggesting that group living is a secondary option to independent reproduction. The Australian allodapine bee *Exoneura bicolor* exhibits a number of traits that occur in cooperatively breeding bird species, including long life-span, repeated opportunities for reproduction, and vulnerability to brood predation and parasitism. The effect of a potentially limiting environmental factor, nesting substrate availability, was experimentally examined as an agent influencing the sociality of *Exoneura bicolor*. We manipulated nesting substrate availability in two separate locations during a time when foundress dispersal is common. No significant difference was found between colony sizes in cases where dispersal options were abundant and cases where dispersal options were limited. We found that provision of abundant opportunities for dispersal did not lead to higher rates of independent nesting, suggesting that cooperative nesting is a preferred strategy regardless of costs of dispersal. Reproductivity per female and brood survival were examined as factors selecting for group living. Low survival of brood in single-female nests has the potential to select for cooperative nesting in this bee.

13-102

THE ROLE OF TERMITE (ISOPTERA) DIVERSITY IN ECOSYSTEM PROCESSES

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Termites are increasingly being recognised as the dominant decomposer insects in tropical ecosystems. However, there are still relatively few studies that explore the complex ecological roles of termites in such systems. For example, we have little idea of the effect and influence of termite diversity on large scale ecosystem processes. In this talk I will outline studies being undertaken as a part of the work of the Natural History Museum's Termite Research Group that attempt to make the role of termite diversity in such processes clearer. I will describe work underway in Cameroon, Congo, Thailand and Malaysia, involving experimental and semi-experimental manipulation of termite assemblages in tropical forests. Special emphasis will be placed on the role of termites in carbon fluxes and decomposition, and how this may be affected by long term historical and short term ecological factors.

13-103

TERMITE SPECIES RICHNESS AND FUNCTIONAL DIVERSITY IN SOUTHEAST ASIAN FORESTS

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Termites are the dominant detritivorous arthropod group in tropical forests, and play a central role as mediators of nutrient and carbon fluxes. However, due to their diverse feeding habits the precise extent of their influence over decomposition processes depends on the functional composition of the local assemblage. A standardized sampling protocol was used to measure termite species richness in primary lowland dipterocarp forests in Thailand, Peninsular Malaysia and Borneo. Differences in species richness between sites is related to latitude while shifts in the dominance of taxonomic and functional groups are discussed in relation to biotic and environmental factors. Changes in the frequency of occurrence of the fungus-growing termites (Macrotermitinae) appears to be influenced by rainfall and moisture levels.

13-105

ANTIBACTERIAL ACTIVITY OF SOME EXUDATES OF ANA-CANTHOTERMES AHNGERIANUS JACOBSON (ISOPTERA: HODOTERMITIDAE)

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The inside of galleries and living chambers of termite nest have special coating consisting of liquid and water-free feces of termites. It seems, the secret of salivary, sternal and other glands may be included in composition of this coating. We reported before, that the chamber coating from natural nest and its analog from artificial nest show the fungistatic activity. The tests were carried out to determine the antibacterial activity of chamber coating analog (the filter paper impregnated with exudates, which are released and accumulated during maintenance of colony in laboratory). The sterile water extracts from termite-treated paper were prepared and sensitivity of the 28 bacterium species to them was tested by agar diffusion method. On the whole the test extracts showed bactericidal effect on the 14 bacterium species including 5 subspecies of entomogenous bacterium *Bacillus thuringiensis* and also bacteriostatic effect on the 2 species. We suppose, that termites *A. ahngerianus* support "sterile" conditions within the nest by own metabolites inclusion in its structure.

13-104

STUDY ON A BIONOMICS OF ASIAN MITE(*TROPILAEELAPS CLAREAE* DELFINADO AND BAKER), MAJOR PARASITE OF HONEYBEES(*APIS MELLIFERA* L. AND *APIS CERANA* FABRICIUS) IN KOREA

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Since the asian mite(*Tropilaelaps clareae* Delfinado and Baker) was introduced from China in 1992, it has caused great damages to Korean beekeeping industry and expanded its distribution. Even worse, it was reported recently that *T. clareae* could overwinter at greenhouse or southern parts of Korea. To set the effective control scheme, a life history of *T. clareae* was examined. After capping of brood cells, eggs were first found in 48-51 hours. The protonymph and the deutonymph were first detected after 66-69 hours and 120-123 hours, respectively. Also, limit of overwintering temperature was studied. According to a survey, *T. clareae* could overwinter above 2°C in Korea.

13-106

MATING SYSTEM IN TWO ANT SPECIES OF THE EUROPEAN SUBGENUS COPTOFORMICA

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Dispersal strategies in ants has been the subject of many studies this last ten years. In the european subgenus *Coptoformica*, male sexuals of at least three species (*F. exsecta*, *F. bruni* and *F. pressilabris*) are characterized by a bimodal distribution of their size (micraners and macraners). In *F. pressilabris*, both morphs, micraners (small males) and macraners (big males), exhibit different behaviours during nuptial flight. Micraners have a significantly higher relative wing surface and relative glycogen content than macraners and may represent the dispersing form. Mating behaviour experiments, including small and big males, show non significant differences in the number of mating attempts when separated. But micraners seem to be more successful in matings than macraners when in competition. Detailed results about mating system of the following two species, *F. pressilabris* and *F. exsecta*, will be presented, including sperm counts of micraners and macraners, sperm transfer in single, double and treble mated females, and the frequency of insemination by both morphs.

13-107

HYDROCARBON PATTERN VARIATIONS IN THE PAPER OF *POLISTES BIGLUMIS BIMACULATUS* NESTS USURPED OR NOT BY THE SOCIAL PARASITE *POLISTES ATRIMANDIBULARIS* (HYMENOPTERA: VESPIDAE)

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In social wasps of the genus *Polistes* nest paper odour is thought to be important for the process of nest and nestmate recognition (Gamboia, 1996).

Comparing the odour of parasitized and non-parasitized colonies, Lorenzi et al. (1996) showed that by the end of colony cycle the hydrocarbon pattern of the two kinds of nest differed: nest paper was covered by alkanes in non-parasitized colonies while it contained both alkanes and alkenes in parasitized colonies.

Fragments of the paper of field colonies of the social wasp *Polistes biglumis bimaculatus* were collected at different moment during colony cycle from both "normal" colonies controlled by the original queen and colonies usurped by the social parasite *Polistes atrimandibularis*. Aim of the present research was to identify the moment in the colony cycle when nest odour begins to vary in colonies usurped by the social parasite. Nest paper fragments were extracted with organic solvent and extracts analysed by GC and GC/MS. Multivariate statistical analyses (PCA and cluster analysis) of the results of the chemical analyses showed that nest odour varies continuously during colony cycle both in parasitized and in non-parasitized colonies. Parasitized colonies do differ from normal colonies regarding nest paper hydrocarbon pattern but parasite-specific compounds (alkenes) appear late in the colony cycle, more than one month after host nest invasion by the parasite queen. The moment when nest paper hydrocarbon pattern of parasitized colonies begins to differ from that of normal colonies correspond to the moment when the parasite brood are at the stage of pupae while host workers are going to emerge.

13-108

Symbiotic Relationship and Genetic Analysis between Ants and Two Epiphytic Myrmecophytes *Hydnophytum moseleyanum* and *Anthorrhiza caerulea* (Rubiaceae: Rubiales) in Papua New Guinea

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The Rubiacerous epiphytic ant-plants which are distributed in/around New Guinea Island belong to Hydnophytinae including five genera. They have the swollen tuber with the complicated cavities. Some arboreal ant species are known to colonize in the cavities. The interaction between ants and the plants is considered as the mutualism because the plants can absorb the nutrients from the debris stored by ants on the cavities through the surface of specialized cavities. However, the ecological interaction of their symbiotic relationship is still unclear. Also, the genetic interaction between ant colonies and the population of these ant-plants are not studied at all.

We investigated the species compositions of ants living in *Hydnophytum moseleyanum* and their territory. The dominant ant was *Philidris* sp. and their colony occupied many ant-plants on plural (mostly two or three) trees. We compared total leaf area of the plants, the number of flowers, of fruits, of seeds, the weight of seeds and the rate of seed germination between the plants occupied and unoccupied by *Philidris* sp. In result, the occupied plants produced bigger seeds significantly and their seeds showed higher germination rate. The ant occupation will be beneficial for their host plants.

The other rubiacerous ant-plant *Anthorrhiza caerulea* was always occupied by the ants of *Dolichoderus* sp. We investigated the genetic interaction of the population of both ants and plants. The result suggested their close relationship.

13-109

THE ROLE OF 9-ODA [(E)-9-KETODEC-2-ENOIC ACID] FOR WORKER DOMINANCE HIERARCHIES IN *APIS MELLIFERA CAPENSIS*

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The presence of the queen in a honeybee colony is perceived by the workers through a blend of fatty acids secreted by the queens mandibular glands. Its major compound (9-ODA) has been shown to inhibit the development of worker ovaries. In queenless colonies this signal is absent, the depression ceases and some workers initiate ovary development. In *Apis mellifera capensis* this process is very swift and as soon as two days after queen loss eggs can be found. Furthermore these workers produce a queenlike pheromone with high 9-ODA levels. We here report on preliminary data of the role of 9-ODA in worker-worker interaction in small experimental groups.

13-110

PHYLOGENY OF THE SWEAT BEE TRIBE AUGOCHLORINI (HYMENOPTERA: HALICTIDAE), WITH IMPLICATIONS FOR SOCIAL EVOLUTION

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Phylogenetic relationships of the genera and subgenera of the New World sweat bee tribe Augochlorini are explored. Eighty characters of adult external morphology are used in cladistic analysis of the thirty-nine recognized genera and subgenera of the tribe (seven of which are new). An attempt is made to include the fossil augochlorine genus, *Oligochlora*, however placement of this taxon is problematic due to the dearth of codable characters.

This phylogeny has implications for the evolution of behavior within this tribe of bees. Both nocturnal foraging and cleptoparasitism have each apparently evolved more than once in the augochlorines. Particularly noteworthy for sociobiology, the primitively eusocial genera - *Augochlora* (*Oxytroglossella*), *Augochlorella*, and *Pereirapis* - form a monophyletic group. Eusociality apparently evolved once in the common ancestor of this complex, with a reversal back to solitary living in *Augochlora* (*Augochlora*).

13-111

NEW DATA ON LARVAL KIN RECOGNITION BY *Apis mellifera* L.WORKERS

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In social insects, kin recognition has been studied most intensively in honeybees and has been treated with different approaches. The common context is the queen rearing because it is one of the few times that workers have an opportunity to control the genotype of future reproductive (Breed et al.1984).This authors found that when worker honeybees were given a choice of rearing queens from related rather than unrelated larvae, the worker didn't prefer to rear sister larvae over unrelated larvae whereas Visscher (1986) obtained positive results with similar techniques.

In our studies, we performed 3 different experiments to test the hypothesis that honeybees can favor closely related individuals when rearing queens. We used colonies of *Apis mellifera mellifera* (dark colored) and *Apis mellifera ligustica* (yellow colored) with queens that were open mated. In a first experiment, worker bees were given the opportunity to choose between one sibling and one unrelated larva grafted in the same cup. We recorded the results by using the color phenotypes of queens after emergence. In a second experiment queenless bees were given the choice of rearing related or unrelated old larvae (4 days after hatching). In a third experiment, we tested the hypothesis that workers selectively feed related larvae by providing them more and/or best quality of royal jelly.

In the two first experiments, workers were unable to make a choice between related and unrelated larvae but in the third experiment one colony of *mellifera* has a tendency to feed favorably the sibling larvae. We are also testing the effect of external resources on the worker's ability to rear selectively larvae.

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VISSCHER P.K., 1986. Kinship discrimination in queen rearing by honey bees (*Apis mellifera*). *Behav. Ecol. Sociobiol.*, 18:453-460.

13-113

QUEEN-WORKER CONFLICT OVER REPRODUCTIVE SEX RATIO AND BROOD MALE RECOGNITION IN THE ARGENTINE ANT

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Inclusive fitness theory predicts that workers enhance their inclusive fitness by biasing reproductive sex ratio towards females. This can be reached by eliminating queen's male offspring. That workers recognise juvenile forms of males is a prerequisite for this assumption. This study tests this possibility. Using queenright societies which elicit elimination of male brood, we regularly took away batches of larvae sorted according to their size. We reared these larvae in queenless societies and we checked sex and number of the resulting pupae in order to determine the ratio of male larvae still alive when the rearing colony status was changed. The results indicate for the first time sex larval recognition followed by male brood elimination. Half of the latter was cannibalised after hatching and the remaining when the male phenotype becomes recognisable. The results are discussed with regards to conflicts concerning sex investment ratio and sexual deception theory

13-112

CHEMICAL AND VISUAL CUES USED BY DEFENDING HONEY BEES TO RECOGNIZE MAMMALIAN PREDATORS

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I collected data from a mass attack of honey bees on a dog and tested the following hypotheses: 1) attacking honey bees target the victim's eyes and oral-nasal area; 2) breath directionally orients the attack; 3) dark colors elicit attack; 4) furriness enhances attack vigor. The highest density of stings on the dog's body were in the anterior muzzle and the eyelids (see Table), a density several times higher than in neighboring areas. Dark areas of the dog's body received over twice as many stings as light areas. Indirect evidence supports the correlation of attack vigor with furriness. The overwhelming role of breath in directing the attack is obvious when 7.8 stings/cm² in the muzzle are compared to 0.02 stings/in² for the feet/lower legs. These

Table — Sting pattern data for dog killed in bee attack

Stings	# Received	Stings/cm ²
Total	3305	
Head	2460	
Face (ears & anterior)	2227	1.8
Anterior muzzle	565	7.8
Eyelid area	79	6.1
Inside mouth	233	
Body (excluding head)	845	0.080
Feet/lower legs	7	0.022
Body stings by fur color		
Black & dark grey	506	0.13
Yellow & yellow-grey	339	0.053

data demonstrate that bees target mammalian breath and eyes, with dark areas and furry textures being auxiliary attracting factors.

13-114

INTRASPECIFIC CLEPTOBIOSIS: AN OPTIMAL FORAGING STRATEGY IN THE NEOTROPICAL PONERINE ANT *ECTATOMMA RUIDUM* ROGER

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Due to their extremely high nest density per hectare (up to 11300), the hunting areas of neighboring colonies of the ponerine ant *Ectatomma ruidum* partly overlap and some workers can enter a foreign nest to rob food reserves. The real ecological impact and function as an efficient foraging strategy of this usual cleptobiotic behavior remain uncertain. Intraspecific food competition between colonies of *E. ruidum* on numerous small and light prey was studied in a coffee plantation in Mexico. For each of the three replications of this experiment, separated by a two-day interval, 1000 dead fruit flies (*Ceratitis capitata*) were used as a unique food source. The species *E. ruidum* realized 90-95% of its exploitation. The predatory activity of each colony, the general kinetics of prey items disappearance and the distances between each colony involved in a cleptobiotic interaction were recorded. The exploitation was done both at a direct and an indirect level, so called "first-hand" and "second-hand" cleptobiosis, with some colonies which never had any direct relation with the food source. Along the three replications, the number of nests exploiting directly the food source did not really change (8, 11, 10 respectively) while the number of nest involved in indirect exploitation increased significantly (8, 11 and 16). The average distance for the direct exploitation of the food source weakly increased between the first and the subsequent repetitions (162 cm vs 196 and 188 cm) while the average distance between the colonies involved in cleptobiosis interactions highly increased during the second and third replication (182, 211 and 253 cm). Such a strategy for the exploitation of an important and unique food source optimizes the foraging efficiency under high nest density conditions. Firstly because direct predation is very high, secondly because the food robbing by ants from the more distant nests on nests located closer to the food source induces an increase of the direct exploitation resulting in a super-exploitation of the food source.

13-115

POLYETHISM IN THE ANT *GNAMPTOGENYS* SP. (FORMICIDAE, PONERINAE)

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Gnamptogenys sp. is an arboreal Ponerine ant with a colony structure in which either queens or gamergates -inseminated, egg-laying workers- can be reproductives.

The division of labour in 1 queenright and 2 queenless colonies of this species was studied through observations of nests with individually marked workers of well known ages from eclosion up to four months.

A clear polyethism among the workers was found in all three colonies, in which two clear categories can be distinguished. A first group of mostly young workers is generally found within the nest, where they are mostly involved in brood care. A second group comprises foragers, that generally represent older workers. An additional intermediate group can be found and is formed by individuals performing a wide range of tasks inside the nest (such as allogrooming, nest maintenance and brood care) while they may also be found outside the nest. Alate and dealate virgin queens can be produced in both colony types, and unexpectedly perform tasks similar to workers like brood care, nest maintenance and foraging.

Although our data suggest that most workers go through an evolution from nurses to foragers, there seem to be no fixed ages of this transition. This suggests that absolute age may not be a good measure in age-polyethism studies for this species. Since in some cases an early specialization to foraging occurred in very young ants, we believe that other mechanisms might influence task choice.

13-117

DIVERSITY OF TERMITES (INSECTA:ISOPTERA) IN PRIMARY TERRA FIRME FOREST IN CAXIUANÃ SCIENTIFIC STATION, PARÁ, BRAZIL.

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Species lists are presented for these area in Amazonian forest. Sixty-four species of termites belonging to 28 genera in three families, were found in a primary terra firme forest, in Caxiuanã Scientific Station "Ferreira Penna" (1° 42'30"S; 51° 31'45" W), Pará, Brazil..

The Nasutitermitinae subfamily especially the genus *Nasutitermes*, were the dominant group in number of species. The majority of the species (36) were wood-feeders, many of them restricted to rotten-wood. The humus-feeders were the second group in number of species (16).

The cohabitation of different termite species in the same nest was more common.

13-116

DOMESTICATION OF THE LEAF-CUTTING ANT: REPRODUCTION OF *ATTA OPACICEPS* BORGMEIER, 1939 (HYMENOPTERA: FORMICIDAE)

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The northeast leaf-cutting ant, *Atta opaciceps* is a widespread species of the Brazilian northeast ecosystems. This attini is extremely resistant to density independent factors such as drought and high temperatures. *A. opaciceps* is also a useful insect which produces: Phytoalexins, organic and mineral fertilizers and a high-quality protein which tastes better than the finest caviar. This investigation aims to elucidate the insect reproductive behavior. It was conducted on a 5ha plot covered with arboreal and arbustive vegetation infested with nests of the formicid in Fortaleza, CE, Brazil from 1991 to 1995. Thirty mature nests, 15-year old and more, were directly and daily inspected for three times, v.g., 09:00, 17:00 and 19:00 GMT. It was disclosed that less than 50% of the nests engage in nuptial flights which last from late December to middle February. This activity starts right after 17:00 and finishes up by 18:15, approximately. From those nests, all produce both male and female in different ratios. Females start flight first and stay from 60 to 80m above the ground level and are followed by males of different nests. They mate during the flight.

13-118

WORKER TASK AND WORKER SIZE OF *AZTECA* ANTS

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Azteca cf. *lanuginosa* (Hymenoptera: Formicidae: Dolichoderinae) is found in the cerrado of Central Brazil where it makes cardboard-like nests on the tallest trees (around 6 m tall). The nests are oval in shape, reaching 33 x 40 cm in diameter, and contain a large number of workers (50,000 to 80,000). There is a continuous variation in size of the workers, with their cephalic capsule width ranging from 0.58 to 1.43 mm. This species is predations on insects, and capture their prey either patrolling actively the plant or waiting for larger prey such as butterflies, beetles and grasshoppers, in coordinated group ambush. Although there is variation in the size of the workers outside of the nest, there is no apparent differentiation in terms of their activities. However the smallest workers (cephalic capsule width between 0.58 and 0.68 mm) were only found within the nest. This result indicates a specialization of the smallest ants to tasks inside the nest.

13-119

NESTMATE DISCRIMINATION IN *LEPTOTHORAX LICHTENSTEINI* BONDROIT ANTS: A KIN-BIASED BEHAVIOR ?

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To test the kin recognition ability of *Leptothorax lichtensteini*, closely related colonies were set up in which the foundress sisters were fertilized by their brothers, and ethological experimentation was carried out in which workers from a colony were transferred into a related or a nonrelated colony. Intruders related to the host colony were licked and participated in trophallactic contacts more frequently, as well as being bitten less frequently than intruders which were unrelated to the host colony. Workers turned out to be capable of recognizing individuals which they had never encountered previously, on the basis of their kinship.

Does kin recognition rank high among the mechanisms whereby the members of a colony recognize each other? A worker which had been removed from its nest for 70 days along with four sisters was subsequently attacked by its nestmates on being returned to its colony. Likewise, a worker which had spent 45 days in an alien colony attacked its own previous nestmates, and vice versa, when the two colonies were brought into contact. In both cases, the workers seem not to have been recognized, as if the genetic cues they carried had been masked by social environment cues.

In this species, recognition among the members of a colony is probably based on a whole complex set of coexisting mechanisms, involving both genetically determined chemical cues and environmental factors (including social factors). The preponderance of one type of cue might vary depending on the context (nestmate or nonnestmate kin recognition).

13-121

HOW DO WORKERS OF THE ANT *FORMICA RUF*A COPE WITH SPATIAL INFORMATION DISRUPTIONS IN THEIR ENVIRONMENT?

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Workers of the ant *F. rufa* are known to use chemical trunk-trails as well as visual landmarks to orient to familiar food sources. However, spatial information disruptions between the two types of cues as well as within visual landmarks can sometimes occur whenever some landmarks are temporarily hidden or permanently displaced by natural agents (fog, wind, etc...) or when they altogether disappear under the action of man. The aim of our study was to investigate the behaviour of ants facing such information disruptions.

In order to work in a fully controlled visual environment our experiments were carried out in the laboratory. The orientation performance of the ants was studied by recording and analysing their trajectories. Previous to all experiments, ants were first allowed to establish a chemical trail between their nest and a permanent food source. In a first series of experiments, no landmarks were placed on the area and the environment was manipulated in three different ways: the whole experimental set up could remain unchanged, be rotated by 180° or surrounded by a white curtain so as to mask the visual landmarks. For each of these situations the chemical trail was either in its usual position or rotated by 90°. In a second series of experiments visual landmarks were placed directly on the area and the location of the food source was associated with one of these landmarks. This particular landmark was then moved at different locations whereas the location of the other landmarks remained unchanged, thus introducing a conflict both between the chemical and visual cues as well as within the visual ones.

Preliminary results show that ants are able to orient successfully when only chemical or visual cues are present but that, on average, the orientation was always better when the two types of cues were present, suggesting the existence of a cross-potential process between the two types of sensory input. The worst performances were observed when the information given by the proximal (within-area) and the distal visual landmarks (experimental room) was reversed.

13-120

DEMOGRAPHIC CHANGES AND TEMPORAL POLYETHISM IN COLONIES OF *ECTATOMMA TUBERCULATUM* (HYMENOPTERA: FORMICIDAE: PONERINAE)

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Ant societies are biologically dynamic structures: their population size and composition change throughout their life cycle. Such demographic fluctuations were examined in mature colonies of *Ectatomma tuberculatum* (Ponerinae) reared under constant laboratory conditions. Three different demographic situations were then characterised by the value of the ratio "workers to brood". In each situation, the behavioural ontogeny of workers was studied from emergence to death. We compared the age at which tasks (i.e. brood care, nest maintenance, guarding the nest entrance, foraging) were first displayed and the rate of their performance. When the ratio "workers to brood" was inferior to 1, workers foraged at the mean age of 22 days old and were engaged over a longer period of time in brood care. When the ratio was superior to 2, the first foraging trip occurred later, at approximately 66 days old, and was usually preceded by a guarding stage. Differences between groups were observed as early as the first week of imaginal life; they mainly concerned alimentary activities and social interactions. It appears that actual colony needs affected by demographic situations greatly influence the behavioural ontogeny of workers. Social stimulations, especially those originating from older nestmates, might partly control the maturation rate of callow workers and as such temporal polyethism.

13-122

EGG-MARKING IN THE BUMBLE BEE *BOMBUS TERRESTRIS*

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In bumble bees "elite workers" often show developed ovaries, but since they do not mate, they only produce haploid male eggs. In the honey bee *Apis mellifera*, the haploid eggs produced by workers are eaten by their nestmates, but those layed by queens are left intact due to an odour marking from the Dufour's gland, which is deposited on the eggs and is recognised by the workers. We investigated whether similar egg-marking occurs in the bumble bee *Bombus terrestris*. Volatile markings were obtained by washing individual eggs in pentane and the resulting odour samples were analysed by gas chromatography and mass spectrometry.

Eggs produced by queens and workers showed significantly different odour bouquets. Furthermore, the volatile patterns were colony-specific. In the odour bouquets, n-alkanes and alkenes were the major classes of compounds. Since the odour composition of the Dufour's glands differed from those of the egg-markings, females of *B. terrestris* obviously mark their eggs with secretions other than only those of the Dufour's gland. Whether the egg-markings of bumble bees encode colony-, caste- or even patriline-specific recognition signals is presently under investigation.

13-123

NEST MATE RECOGNITION OF WORKERS AND THEIR HYDROCARBON PROFILES IN JAPANESE, ASIAN AND EUROPEAN HONEYBEE.

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The Japanese honeybee (*Apis cerana Japonica* Rad.:Acj) and the European honeybee (*Apis mellifera* L.:Am) share the same habitat in Japan. Workers of both species were experimentally introduced to their own hive, to different hives of the same species, or to hives of the other species. Am hives accepted 52% of the trials of day-0 Am workers and 87% of the Acj foragers. On the other hand, Acj hives accepted almost all foragers from the other hive (96% average) and day-0 Am workers as well (90%). Hive mates as well as non-hive mates accidentally damaged during experiments were rejected, even in the case of the friendly Acj. As a result, nest mate recognition in Acj among different colonies and against different species was milder than that of Am.

Hydrocarbons were analyzed by GC and GC/MS, and were compared among 3 species, using wax and body surface extracts from workers and drones. Each species was distinguishable not only by a combination of components, but also by occurrences of specific components. Acj workers contained C₂₃, C₂₅, C₂₇ and Am workers contained C₂₅, C₂₇, C₂₉, C₃₁ and C₃₃ with various degrees of unsaturation (0 to 3). Alka-6,9-dienes (C₂₅ to C₂₉) was present in Acj, while Am possessed dienes with (CH₂)_n (n > 3) between double bonds.

Hydrocarbon profiles (HCP) among individual workers and drones of a hive or with age were different in Acj, Am in Japan and Asian bee (*Apis cerana indica*) in Nepal. HCP of workers were divided into groups of 3 to 10, whereas those of drones were divided into groups of 1 or 2. These results could be explained by queen's multiple mating and/or inbreeding in honeybee society and suggest that these drones are homozygotes.

13-125

WHAT ROLE FOR KIN SELECTION IN COMMUNAL BEE SOCIETIES? THE CASES OF SOME EUROPEAN ANDRENIID BEE SPECIES (HYMENOPTERA: ANDRENIDAE)

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Bees (Apoidea) exhibit a wide diversity of social organizations and they therefore provide suitable material with which to test theories for the evolution of sociality. One such theory, invoking kin selection, has been the major paradigm for understanding the evolution of sociality in the Hymenoptera because of the inherent asymmetries of relatedness in eusocial societies of ants, bees and wasps. Yet rarely has relatedness been measured in facultatively social species, those in which one can measure the factors favouring the evolution of sociality.

We examine the potential importance of kin selection in facultatively communal bee species, that is, species with amongst the lowest levels of social organization within Hymenoptera involving little more than the use of a shared nest entrance. We do so by measuring genetical relatedness of nestmate females using microsatellite DNA markers. Estimates of relatedness in established nests are generally low, and not significantly different from zero.

Inclusive fitness benefits may nonetheless be important in nest initiation, when small numbers of females join together to construct a communal nest. Field manipulative experiments suggest that, even in this situation, kin selection is of little importance in communal bee societies.

13-124

A GENERAL APPROACH TO THE VISUALISATION AND ANALYSIS OF DOMINANCE INTERACTIONS: THE PONERINE ANT *GNAMPTOGENYS* SP. AS A CASE STUDY

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Dominance hierarchies are traditionally represented using dominance matrices and statistically analysed using Appleby's test for linearity. These methods were however mainly developed for species where the repertoire of agonistic interactions can easily be pooled together and clear-cut conclusions on the outcome of encounters are obvious, e.g. in chickens. Dominance orders are however also known to occur in various social insects. In the primitive ponerine ants where actively reproducing inseminated workers (gamergates) occur, evidence for dominance-regulated reproduction has recently accumulated. However, the use of dominance matrices implies the reduction of temporal and sequential information of interactions. Also, additional data on the difference in status of the interacting individuals can be deduced from the reaction (aggressive vs. submissive) of the aggressed individual. As a model for our research on improving the analysis, the arboreal ponerine ant *Gnamptogenys* sp. of Sulawesi was used. A generally applicable computer program was developed allowing a visual representation of interaction-type data-sets. An automated representation of the interactions between all individuals is obtained, with the simultaneous display of type of action and the type of reaction. This approach has many advantages: apart from giving a perfect representation of temporal dynamics of hierarchies, it provides a framework for gaining insight in factors which have so far not been considered in research on dominance hierarchies in ants. Currently, attempts are made to include this additional data in the analysis in order to maximise the correlation between egg-laying rates or ovary development and the obtained dominance hierarchy - models which could then be tested on different colonies. Ultimately we hope to provide a tool for assessing the most accurate picture of the dominance hierarchy based on interaction data.

13-126

ACTIVITY CYCLES OF EXOCRINE GLANDS OF *BOMBUS TERRESTRIS*Monica Brozzoni¹, Hayo Velthuis², Marie José Duchateau², Johan Billen³ and Eric Schoeters³

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Bumblebees use pheromones in sexual (Kullenberg et al., 1970; van Honk et al. 1978) as well as in dominance communication (van Honk et al. 1980; Röseler et al. 1981). Two glandular systems are involved, the labial and the mandibular gland.

We studied the changes of the glandular morphology during the aging of males and queens, using light and electron microscopic techniques. While both glands are present in either sex, only one has prominent secretory activity. In the male it is the cephalic labial gland, in the queen the mandibular gland that demonstrates (ultra)structural changes related to the secretion of pheromones.

The labial gland is composed of many acini made up of polygonal cells. The acini are empty in newly emerged males but they are totally filled with secretion at 5-10 days of age, so they become very much enlarged while the cells flatten. Later (15-30 days old males) the gland is not producing pheromones any more, the acini become smaller again due to the discharging of the secretion and the cytoplasm attains a vacuolar disrupted appearance.

The mandibular gland of the queen shows two distinct periods of activity: the first coincides with the mating age, before hibernation, the second with the development of the colony. In between a reactivation of the secretory cells takes place. The ultrastructural changes are very obvious near the site of the end apparatus. At the end of the colony cycle we observed clear sign of degeneration of the secretory cells, such as the occurrence of primary and secondary lysosomes together with lamellar inclusions.

13-127

CHEMICAL 'DISGUISE' BY THE QUEEN OF THE SLAVE-MAKING ANT *POLYERGUS RUFESCENS* DURING COLONY FOUNDATION.

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Polyergus rufescens is an obligatory slave-making ant species that depends entirely (for food, brood rearing and colony maintenance) on the slaves species *Formica cunicularia*. After mating, the newly fecondated *Polyergus* queen enters a *Formica* nest where she kills the host queen, appropriates the brood and gets adoption by adult workers. To understand how the queen *Polyergus* can introduce into the *Formica* colony, the cuticular hydrocarbons of the queen (before and after usurpation) are compared to those of the slaves (queen and workers) living in monospecific or mixed colonies. It appears that the pattern of the *Polyergus* queen before entering the *Formica* nest, differs from that of the *Polyergus* queen after usurpation. Before usurpation, the *Polyergus* queen has small amount of cuticular hydrocarbons. It seems that the slave-keeping queen synthesizes the minimal necessary amount of her own cuticular hydrocarbons. Chemical changes are observed when the queen enters the slave's nest where she obtains the cuticular hydrocarbons from the slaves which keep their original pattern. In fact, the cuticular profile of the queen *Polyergus* becomes more similar to that of the monospecific *Formica* queen. We will discuss on this parasitism strategy.

Section 14

Apidology and Sericulture

14-001

NEUROPEPTIDES REGULATING METAMORPHOSIS OF THE SILKWORM, *BOMBYX MORI*

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Insect molting and metamorphosis are regulated by various neuropeptides. During past 10 years, these neuropeptide, such as prothoracicotropic hormone, eclosion hormone, and bombyxin have been purified from the silkworm, *Bombyx mori* and chemically characterized. Furthermore, the cDNAs and/or genomic DNAs of these hormones have cloned and sequenced. In this talk, Our research on these neuropeptides is reviewed.

14-002

ECDYSTEROIDS AND JUVENOIDS CONTROLLING GROWTH AND DEVELOPMENT OF THE SILKWORM *BOMBYX MORI*

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Numerous pioneers of insect endocrinology used the silkworm *Bombyx mori* as a model insect for the studies of the control of post-embryonic development and reproduction. In 1900 an organ was described in the larva only. This organ was termed "prothoracic gland" by Ke (1930) but its function was elucidated by S. Fukuda in 1940. During this decade J.J. Bounhiol (1936) published several papers on the role of corpora allata in the onset of metamorphosis. During a long period the function of these different glands was only revealed by removal or implantation of the gland at various periods of the development. We had to wait until 1963 to have the ecdysone chemical structure by Karlson *et al.*, and 1967 to obtain the first insect juvenile hormone structure by Röller *et al.*. As soon as chemical elucidation occurred it became possible to develop techniques for hormone titration. With such tools, hormone fluctuations during the development were established. From this period, numerous studies were achieved to determine the factors that regulate the balance between the different hormones. Both *in vivo* and *in vitro* studies gave information on the responsiveness of the glands. Important data were obtained on the different routes in biosynthesis and metabolism of hormones. Ohnishi and his group purified several ecdysteroids in silkworm ovaries and in embryos but their role remains to be clarified. Molecular approaches and genetics are an important means for clarification of the regulation of the development. The elucidation of the hormone structures and available biological assays allow the discovery of hormone analogs that were suggested to be used as insecticides (Williams 1967) or in sericulture.

14-003

DIAPAUSE HORMONE OF THE SILKWORM, *BOMBYX MORI* (LEPIDOPTERA: BOMBYXIDAE)

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The silkworm, *Bombyx mori* enters diapause at the early embryonic stage. Diapause is a physiological phenomenon controlling the life span, so that many physical, chemical and biological informations are used to determine diapause character. Diapause hormone (DH) plays a central role for induction of diapause, and the secretion of DH is controlled by temperature and photoperiod experienced at maternal stages.

The temperature function is elucidated by measuring the DH gene expression at 15 °C and 25 °C conditions. A high temperature induced DH gene expression at the specific stages of embryonic and post-embryonic development. This temperature stimulus provided a high coefficient value between DHmRNA content and diapause egg incidence. Diapause hormone enhances trehalase activity in developing ovaries, that is the initial biochemical process leading to diapause metabolism. An RT-PCR determination of trehalase mRNA in ovaries clearly demonstrated that DH induced trehalase gene expression as early as 1 h after hormone treatment.

The recent advances on regulation mechanisms of DH gene expression and molecular action of DH will be considered.

14-004

PEPTIDES REGULATING PHARATE LARVAL
DIAPAUSE OF *ANTHERAEA YAMAMAI*

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By ligation experiments, this study ascertained that a repressive factor (RF) produced in the region of the mesothorax (Suzuki *et al.*, 1990, 1994), is indispensable for diapause maintenance in pharate larvae of the wild silkworm, *Antheraea yamamai*. To identify the factor, extracts from prothoracic glands, mesothoracic ganglia, suboesophageal bodies and the mesothoracic part of the foregut were compared with *Bombyx* diapause hormone in a diapause termination bioassay. Only the foregut extract delayed, and in a double application assay, prevented diapause termination. This RF activity was heat stable and destroyed by proteinase. By analogy with Bom-DH, I propose that RF is named Any-diapause hormone. This study also identified a maturation factor inducing post-diapause development, using the methods practiced in the silkworm for eclosion hormone. This peptide-like hormone and Any-DH are different from the FXPRL amide peptides by some results of bioassay. Then a complete isolation of these two hormones is necessary to understand the mechanism controlling diapause in insect pharate larvae.

14-006

IN VIVO AND IN VITRO ANALYSES OF FENOXYCARB
INTERACTION WITH THE ENDOCRINE SYSTEM OF THE
SILKWORM, *BOMBYX MORI*.

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Fenoxycarb, an insect growth regulator with juvenile hormone (JH) mimic effects, acts at very low doses on last-instar larvae of the silkworm, *Bombyx mori*. Last-instar larvae treated with only some picograms of fenoxycarb are unable to spin their cocoon and to undergo metamorphosis, but stay as dauer larvae and continue to feed. JH and its classical analogs are known to delay or inhibit spinning and pupation at higher doses in the silkworm. Such chemicals prevent normal commitment to metamorphosis by inhibiting ecdysone production in the prothoracic glands and PTTH release from the brain-corpora cardiaca-corpora allata complex. Fenoxycarb does not involve a modification in JH production since corpora allata ablations do not restore the normal development of treated larvae. However, Enzyme Immuno Assay(EIA)-detected ecdysteroid peak, which triggers spinning and pupation in normal larvae, is absent in permanent larvae. Therefore, fenoxycarb seems to interfere on mechanisms controlling ecdysteroids titers in last-instar larvae, as does JH. In vitro cultures of prothoracic glands will permit to determine whether fenoxycarb, like JH, can inhibit ecdysone production by acting directly on the gland, or at an upstream step, on the PTTH-producing neurosecretory cells. In addition, a highly sensitive EIA for fenoxycarb is being prepared in order to establish the kinetic of its activity.

14-005

EFFECTS OF TEBUFENOZIDE, AN IGR WITH ECDYSTEROID-LIKE ACTION
ON THE SILKWORM *BOMBYX MORI* L.

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In spite of the large number of insecticides available for pest protection, the search for new insect control agents is continuing. Among the approaches to develop new safer insecticides, the development of insecticides based on the insect endocrine system is greatly improved. Numerous hormone agonists have been synthesized, but only a few have been developed into useful insecticides. Recently a non-steroidal ecdysone agonist (Tebufenozide) has been registered as an insecticide (Confirm^R or Mimic^R). According to the very high sensibility of the silkworm to the IGRs possessing JH activity, we first improved the sensitivity of the silkworm against this IGR possessing an ecdysone-like activity. The ED50 is around 1µg/larva, a value too high to act as a contaminant on the mulberry leaves. Then the Tebufenozide action was evaluated on different ecdysteroid-regulated biological systems and compared with the effects of the ecdysteroids. The first biological event observed after tebufenozide administration was the arrest of feeding followed by the apolysis. The treated worms were unable to molt and they died in their unshed larval cuticle. As with ecdysone, tebufenozide elicits wing disc evagination. We never observed the beginning of spinning but the posterior region of the silk glands revealed precocious histolysis quite independently from the developmental stage of the larvae. The dauer larvae induced by JH mimic treatment do not undergo an extra larval molt if injected with Tebufenozide. We also evaluated the effects of tebufenozide on growth and maturation of ovaries since it was demonstrated that in silkworms ecdysteroids replace the function of juvenile hormones.

14-007

DIFFERENTIAL SYNTHESIS AND UPTAKE OF
STORAGE PROTEIN (SP) BY VARIOUS FAT BODY
TISSUES DURING DEVELOPMENT OF *BOMBYX MORI*

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Insect fatbody is a versatile tissue performing myriad of functions. Depending on their locations, the tissue has been traditionally recognised as peripheral and perivisceral and frequently compared to mammalian liver and adipose tissue. Since structural changes take place rapidly during larval development, it is difficult to distinguish between regional or developmental differences. Recently, in 1992, it was established that in *Heliothis zea* the fat body that turns blue, due to the sequestration of the blue colored storage protein, is the perivisceral tissue that is assigned the role of storage and the colorless peripheral tissue is the synthetic tissue. The punch line of their report was a fact that such specialised tissues exist in other insect species that lack a colored storage protein. Our search in this direction in *Bombyx mori* revealed that fatbody tissues at different locations differ in their morphology from the last instar stadium through adult life. Our *in vitro* and electrophoretic studies revealed the differential storage protein synthetic capacities of these tissues and the specialised organ of storage during prepupal stages in *B. mori*. Studies pertaining to the levels of SP mRNA transcripts in different fat body tissues are under progress.

14-008

EXTRACTION, CLONING AND SEQUENCING OF DNA FROM *NOSEMA BOMBICIS*X. Y. ZHANG, K. M. LU, C. X. Zhunag, W. M. Hu, X. Y. Xu, Z. R. Huang¹ and H. S. Chen²

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This article reports the experimental results of extraction, cloning and partial sequencing of *Nosema bombycis* DNA. The total nucleic acids were extracted from purified *N. n.* spores by SDS-proteinase method. After digested with RNase and DNase, the ethidium bromide stained pattern of agarose electrophoresis showed *N. b.* spore's nucleic acids contain a high molecular weight of RNA. The *N. b.* spore DNA cleaved with restriction endonuclease EcoR I and Hind III were demonstrated smear pattern in AGE. Using plasmid pTZ18R as vector, *Escherichia coli* DH5 as host cell, the EcoR I cleaved DNA of *N. b.* were cloned by shot gun method. Four recombinant plasmids were screened and isolated. They were temporarily named as pTZ18RN. b. 1, pTZ18RN. b. 2, pTZ18RN. b. 3 and pTZ18RN. b. 4. The insert DNA size in the four recombinants were 1.0, 1.2, 1.8 and 1.9kb respectively. The DNA fragments were labelled with ³²P-dCTP by random primer labelled method as a specific probe. Hybridization of these probes with the DNA of *N. b.* spore, MG1, *Nosema cyathia* and *Nosema phyllostactica* showed strong hybridization signals only in *N. b.* spore DNA. A Part of insert DNA fragments were sequenced with dideoxy chain termination method.

14-010

CHITIN FROM SILKWORM: ITS PREPARATION AND APPLICATIONS

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Chitin from erisilkworm, *Philosamia cynthia ricini* and silkworm, *Bombyx mori* was prepared from larvae and pupae. The mild procedure was tested and chosen for the deproteinization and decalcification of the cuticles, since it minimizes both chain hydrolysis and deacetylation.

As a natural polymer, chitin is a ideal medical macromolecular material. Chitin fibers having suitable denier and higher tensile strength are produced by wet-spinning and finishing. A chitin suture for medical use is made with such fibers. It has the advantage of histocompatibility and can be degrades and absorbed in vivo.

A system of subdermal implants that release a steady dose of levonorgestrel for long term, reversible contraception is being developed. It consists of a biodegradable chitin capsule with a rod composed of the drug and chitin.

Chitin and its derivatives exhibited antigenic activity against bacteria and fungi. They are successfully used for freshness preserving of some kinds of fruits and vegetables.

14-009

TRANSFERRING SYNTHETIC ANTIBACTERIAL PEPTIDE GENE FROM CHINESE OAK SILKWORM INTO MULBERRY PLANT BY USING TI PLASMID FROM *AGROBACTERIUM TUMEFACIENS*Z. W. Guo, Q. J. Zhang, C. X. Zhuang, Z. R. Huang¹, A. Y. Chen, L. W. Kong².

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Transferring mulberry plant, *Morus alba*, by infection of Ti plasmid from *Agrobacterium tumefaciens* was studied. Plants selected on kanamycin containing medium were confirmed to express the introduced 35S-synthetic antibacterial peptide gene (ABG) from Chinese oak silkworm, *Antheraea pernyi*. The introduced gene was obtained by fusion of CaMV 35S promoter with ABG. Using the kanamycin resistant gene in Co24 plasmid, the mulberry leaf discs of adventitious bud formed from infected tissues were selected on the MS medium containing kanamycin at 25µg/ml. When they were transferred to the selection medium at 4-5 days, the nopal synthetase activity was detected by electrophoresis. The transformation was confirmed in 40 days with kanamycin resistance by DNA dot blotting. The results showed the synthetic antibacterial peptide gene was transferred into mulberry plant.

14-011

COMPARATIVE ULTRASTRUCTURES OF COCOON FILAMENT FORMATION IN SILK SPINNING INSECTS

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Ultrastructures of the liquid silk materials and cocoon filaments of *Bombyx mori*, *Antheraea yamamai*, *Antheraea pernyi*, *Antheraea mylitta*, *Antheraea assama*, and *Attacus atlas* were studied by both transmission and scanning electron microscopy. In *Bombyx mori*, the elementary fibroin fibers in the fibroin globules in the gland cells are released into the silk layer by exocytosis, forming masses of tangled fibroin fibers 1-5 µm in diameter; these are stored in the silk layer for 30 to 60 minutes. Lysosomal materials, frequently discharged into the silk layer from the gland cells, are completely digested in this layer without transfer into the lumen, passing through the outer cuticular membrane. Therefore, the fibroin column is composed solely of masses of fibroin fibers.

All of the wild silkmots mentioned above, however, have no silk layer because there is no cuticular intima. Therefore, the elementary fibroin fibers in the fibroin globules are directly released into the lumen by exocytosis and form small masses of fibroin fibers 0.5 to 1 µm in diameter, then accumulate on the surface of the fibroin column in the central lumen. During the maturing and spinning periods, numerous lysosomal materials are released into the fibroin column. By digestion they become various sized vacuoles ranging 0.5 to 5 µm in diameter, and all contain small amounts of fine fibrous materials. The fibroin column is thus a mixture of the masses of fibroin fibers and the vacuoles.

In TEM cross sections of *Antheraea mylitta*, the columnar fibroin contains more than 4,000 vacuoles (from 1 µm diameter up) and also many vacuoles of small size. Shapes of the vacuoles change greatly from globular to long tubular shapes in the space between the middle and the anterior silk glands. In the cross section of cocoon filaments of *Bombyx mori*, the filament is rather triangular in shape, and there are no vacuoles in either fibroin or sericin areas. Most of *Antheraea* insects have numerous tubular vacuoles in their cocoon filaments, although the numbers differ by insect species.

14-012

MECHANISM OF LIGHT INDUCED GREEN COLORATION OF THE COCOON IN THE JAPANESE OAK SILKWORM, *ANTHERAEA YAMAMAI*

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The cocoon of the Japanese oak silkworm, *Antheraea yamamai*, is green in nature, whereas it is yellow in darkness. Previously, we revealed that this phenomenon is based on light irradiation at pre-spinning stage, and light acts directly on the hemolymph to bring an accumulation of "cocoon blue pigment" in the silk-gland. In the present work, HPLC analysis of the larval hemolymph showed the followings: (a)the larvae reared in darkness did not contain the blue pigment in their hemolymph, (b)after light irradiation on the larvae for 60 minutes, the pigment appeared in the hemolymph, (c)light irradiation on the isolated hemolymph also caused the formation of the pigment, (d)light irradiation before gut-purge was not effective for the formation of the pigment.

14-014

A NEW COMPONENT OF THE SILK FIBRE PRODUCED IN THE MIDDLE AND POSTERIOR SILK GLANDS

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Analysis of water-insoluble cocoon proteins of the wax moth, *Galleria mellonella*, revealed presence of an 18 kDa component that could not been identified with known silk proteins. An oligoprobe derived from partial N-terminal sequence of this protein was used to screen a cDNA library prepared from the silk gland RNA. A clone was isolated that encodes a protein of 166 amino acids, including the N-terminal region identified by cocoon protein sequencing. The protein differs from all known constituents of lepidopteran silk. With the use of the cDNA clone we found that the corresponding gene, called *seroin*, is expressed in the middle and, to a lesser extent, in the posterior silk glands, but in no other tissue. The content of seroin mRNA in total RNA is high in the silk glands of feeding larvae, declines at the time of larval ecdysis, and reaches a maximum during cocoon spinning. Expression of the *seroin* gene is apparently hormonally regulated: application of a juvenoid to mobile prepupae prevented the drop in seroin mRNA content associated with pupation, whereas application of 2 µg 20-hydroxyecdysone to explanted silk glands caused an abrupt decline of the content.

14-013

PURIFICATION AND CHARACTERIZATION OF INSECTICYANIN FROM THE LARVAL HEMOLYMPH OF *RHODINIA FUGAX* (LEPIDOPTERA: SATURNIIDAE)

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Fifth-instar larva of the saturniid silkworm, *Rhodinia fugax*, resembles a leaf of the host plant, *Quercus serrata*, in color and shape. The spectral reflectance of the larval integument of *R. fugax* corresponded with that of *Q. serrata* leaf. To examine the relationship between the biliverdin-binding protein and the larval coloration of *R. fugax*, blue biliprotein, (insecticyanin, INS) was isolated from the hemolymph of fifth-instar larvae using hydrophobic interaction chromatography and ion-exchange chromatography. The purified INS showed a native molecular weight of approximately 37,000 in gel-filtration, whereas the apoprotein was found to be a single polypeptide of a Mr of 27,000 in SDS-PAGE. The blue coloration of INS was due to the presence of biliverdin relative pigment, which was non-covalently bonded with the apoprotein. N-terminal sequence of *Rhodinia* INS showed about 40% homology to other biliverdin-binding proteins, such as *Samia* BBP, *Pieris* BBP, *Manduca* INS and *Agrius* INS. *Rhodinia* INS was found not only in the larval hemolymph but also in the epidermis and cuticle. These results suggest that INS plays an important role in the protective camouflage in *R. fugax*.

14-015

PRIMARY STRUCTURE OF WILD SILKMOTH FIBROINS ESTIMATED FROM NUCLEOTIDE SEQUENCE

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It is of interest to study how different types of fibroin are distribute in wild silkmoths and utilize commercially. According to X-ray diffraction analysis, *Antheraea* and *Philosamia* fibroins belong to β(3a) and β(3b) types, respectively. We already reported that fibroin genes of Japanese oak silkworm, *Antheraea yamamai*, and Chinese tusser silkworm, *Antheraea pernyi*, were cloned and partially sequenced. The comparison of amino acid sequence deduced from the coding region between two silkworms shows that the both fibroins consist of two types of motif following poly alanine sequence. We analyzed the gene structure of other wild silkworm fibroins by southern blotting and found that there are significant homology among these fibroin genes. This result strongly indicated that there are some relationships concerning to the origin of fibroin genes. In this presentation, we report the summary of these recent data and possibility of further works using wild silkmoth fibroin genes.

14-016

UTILIZATION OF A CELL LINE ESTABLISHED FROM CHINESE OAK SILKWORM FOR PLAQUE-PURIFICATION OF THE *ANTHRAEA PERNYI* NUCLEAR POLYHEDROSIS VIRUS AND CONSTRUCTION OF A NOVEL BACULOVIRUS EXPRESSION VECTOR SYSTEM

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In the baculovirus expression vector system using the *Bombyx mori* nuclear polyhedrosis virus (BmNPV), expression of some proteins is much higher in silkworm larvae than in *B. mori* cell lines. Therefore, it is expected that the similar or much higher level of foreign gene expression would be obtained using the giant wild silkworm, *Antheraea pernyi*, and *A. pernyi* NPV (ApNPV). Additionally, pupal diapause of *A. pernyi* is an advantage over *B. mori*, because diapausing *A. pernyi* pupae can be stored for several months until infecting them with recombinant ApNPVs. Recently, a cell line, NISES-AnPe-428 (AnPe), was established from *A. pernyi* embryos. Using this cell line, we started to construct an ApNPV vector system and obtained following results.

1. We demonstrated that AnPe cells are susceptible to ApNPV. It was also demonstrated that AnPe cells do not support the BmNPV replication and that BmN4 cells derived from *B. mori* do not support the ApNPV replication.
2. Three clones of ApNPV were plaque-purified using AnPe cells. When DNA genomes of these clones were digested with restriction endonucleases, some differences in the electrophoretic profile among their restriction fragments were observed.
3. Diapausing *A. pernyi* pupae were infected well with each ApNPV clone. It took more than one week before the pupae infected with each clone began to die and within 25 days after infection all the infected pupae died.
4. The polyhedrin gene of ApNPV was successfully identified by the Southern hybridization using genomic DNA of ApNPV clone A and a DNA probe containing the polyhedrin gene of BmNPV. The 6.6 kbp *Pst*I fragment containing the polyhedrin gene of ApNPV was cloned.
5. Partial DNA sequences of the ApNPV polyhedrin gene and its flanking regions were determined.

Construction of a transfer vector plasmid is now in progress.

14-017

ON THE INHERITANCE OF NEW COCOON COLOR "EMERALD GREEN (EG)" IN THE JAPANESE OAK SILKWORM, *ANTHRAEA YAMAMAI*

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A new cocoon color strain of Japanese oak silkworm was found in our laboratory and it has been maintained as "Emerald Green (EG)" strain. It is the first report of spontaneous mutant in this insect. The cocoon of this strain appears to be more bluish green colored than that of normal strain "Normal Green (NG)". By earlier study of biochemical analysis, it is recognized that the normal strain has yellow and blue pigments. This yellow pigment is originated in the plant leaves as food and it is transferred into the silk gland of larvae, and the blue pigment is synthesized in vivo under light condition.

We clarified a deficiency of yellow pigment in EG cocoon, by the method of biophysical colorimetry. As a result of progeny test after crossing between two strains, we confirmed the genetic control by a single recessive gene, and the statistical inference by use of cluster analysis was carried out to make clear the variance component within and between phenotypes in the color difference among individuals.

14-018

A METHOD IMPROVED FOR ARTIFICIAL HATCHING IN EGGS OF THE WILD SILKMOTH, *ANTHRAEA YAMAMAI*

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The method of artificial hatching with an imidazole derivative KK-42 has been developed in eggs of the wild silkworm, *Antheraea yamamai* and in the gypsy moth, *Lymantria dispar japonica*. Particularly this compound was effective to break diapause of pharate larvae in the intact eggs and in the naked pharate larvae removed from chorions. In the latter case, KK-42 concentration was 100-fold lower (0.1 µg/pre-larva) than in the former. KK-42, however, was sometimes harmful to induce died individuals. To overcome this point, we examined some imidazole derivatives to break pharate larval diapause. As a result, TH-27 was unharmed and effective for diapause breakdown in naked individuals.

Additionally, we analyzed the mode of action of imidazole derivative in diapausing and diapause-terminated pharate larvae. This study can indicate a model of its function.

14-019

THE SATRUNIID INSECT IN NEW ZEALAND

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At present, three species of Satruniid insect are known living in New Zealand. But originally, it is considered that there are no live under the native condition in this country. However about 1920s one of Satruniid, Gum emperor moth (*Antheraea eucalypti*), was looked and immediately it has spread throughout the north Island and in south Island. This caterpillar feeds on leaves most varieties of eucalyptus and also on pepper tree. Now, it is most popular moth in this country.

The other one is north american silk moth, cecropia (*Hyalophora cecropia*). This insect was found as a caterpillar feeding on a apple leaves in a part of north Island in first of 1980s. And it is consider as feeding on a wide variety of plants so the absence of its natural enemies. The next one is new species of silk moth, *Samia cynthia*, it found at the cocoon stage on the chinese tree of heaven in Auckland city. On these two silk moths, the Ministry of Agriculture & Fisheries of New Zealand has great interest therefore they have able to pest insect on orchards in this country. In this time, the author report mainly on life style of Gum emperor caterpillar.

14-020

APPLICATION OF SILK-SPINING INSECTS TO EDUCATION AND WELFARE
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- 1.Application to education
It can help the intellectual curiosity and the scientific reserch spirit bud out in infants to use bombyx silkworm and wild silkmooths as teaching materials for infant education.
- 2.Application to welfare
In the welfare facilities where there are people advanced in age or suffering from dementia, it can be applied to the activities for their worthy life. The bombyx silkworm, which used to commune with people, can help dementia people refresh their memories and find out joy and support to live.
- 3.Application to the conservation of nature and the propulsion of tree planting
According to the breeding study of *Attacus atlas* done in the welfare facilities for the aged, the results of scientific reserches of its ecology and the development of artificial diet make a contribution to protection and preservation of *Yonagunisai*, a natural monument in Okinawa prefecture. With this approach to protection and preservation of the insects in enviromentally critical condition in the facilities for the aged who are the social weak, we can give to children a welfare education for understanding of the significance of life as well as the enviromental education. It will lead to the combined education of welfare and enviroment. Also it is useful for cooperation in the study and industrialization on *Attacus atlas* in Indonesia. And it would give a contribution to tree planting of necessity for planting to breed it.

14-022

STUDIES ON CERTAIN ASPECTS OF MUGA SILKWORM (*ANTHERAEA ASSAMA* WESTWOOD) CULTURE ON DIGHLOTI (*LITSAEA SALICIFOLIA* ROXB.)

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The unique natural silk fibre golden yellow muga is produced by muga silkworm (*Antheraea assama* Westwood) which is exclusively produced in Assam, a state of North-Eastern Region of India and nowhere else in the world. The polyphagous muga silkworm is mainly reared outdoor on som (*Machilus bombycina* King) and soalu (*Litsaea polyantha* Juss). Dighloti (*Litsaea salicifolia* Roxb.) has certain advantages of muga silkworm rearing that is due to bushy and evergreen nature of the plant and good response to pruning. Despite tremendous potentialities, the plant has been neglected so far as a food plant of muga silkworm.

The present paper highlights the comparative biochemical composition of the leaves and rearing performance of the muga silkworms on the plant. The findings of the study may help in establishing it as an important primary food plant of muga silkworm both for outdoor and indoor rearing.

14-021

THE DISTRIBUTION OF SILK PROTEIN/PEPTIDES IN THE SILK GLAND REGIONS OF MUGA SILKWORM *Antheraea assama* DURING DEVELOPMENTAL STAGES
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Like any other proteins, silk protein is also a polymer of aminoacids. It is composed of two kinds of protein-Sericin and Fibroin. During the fifth instar, fibroin synthesis occurs in large amounts in the posterior silk gland and then translocated into the middle silk gland where it is stored. Recent analysis of the silk gland protein during IVth to Vth instar by SDS-PAGE has revealed 6-19 bands within 24-67 Kda. Differential peptide synthesis was studied with late fifth instar larvae. Anterior silk gland gives ten peptides which are equally distributed in both low (24-45 Kda) and high (45-67 Kda) molecular weight regions. Both middle and posterior silk gland exposed 12 bands at high molecular wt region where as 3 bands were detected at this region for posterior silk gland. Among all three regions of the silk gland, middle one showed the presence of the heaviest peptide. Analysis of the silk gland excluding the anterior part at 24 Hrs after spinning revealed 7 peptides in the same positions as observed for middle and posterior gland and other 4 peptides were detected at high molecular wt region. The significance of this studies will be discussed. (Supported by Department of Biotechnology and Central Silk Board, Govt.of India)

14-023

DETERMINATION OF SUSCEPTIBLE AGE FOR COLD TREATMENT FOR THE EXTENSION OF PUPAL DIAPAUSE IN TROPICAL TASAR SILKWORM, *ANTHERAEA MYLITTA* DRURY.

BY
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A B S T R A C T

Bivoltine brood of Indian tropical tasar silkworm *Antheraea mylitta* Drury (Lepidoptera:Saturniidae) undergoes pupal diapause from mid November to mid June (passing tropical winter and summer). A substantial portion of the adults start emerging from 1st week of June. However, due to severe summer up to 3rd week of June, the rearing of larvae hatched from eggs laid by these females is uneconomical. To overcome this problem, it is imperative that the adult emergence is delayed up to the onset of monsoon (around 20th June), when the atmosphere cools down enough to rear the larvae profitably. In the present study diapausing pupae of various ages (60 to 175 days) were subjected to low temperature (7±2°C) treatment for 15 days. The response was most pronounced on the pupae having age between 150 to 165 days as the peak adult emergence was delayed by 15 days. Pupa of ages less than 120 days did not respond to the treatment. The findings were further substantiated with biochemical assessment which is reported.

14-024

AGE-RELATED CHANGES IN COUPLING APITUDE, OVIPOSITION BEHAVIOUR AND FERTILITY IN THE FEMALE MOTH OF *ANTHRAEA MYLITTA* D. (LEPIDOPTERA : SATURNIIDAE).

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ABSTRACT

Reproductive senescence was studied in the short-lived species *Antheraea mylitta* D. Virgin female moths of various ages (in days) were mated with newly eclosed males (day 0) for 8 h. under prevailing conditions of temperature and humidity. The coupling aptitude (i.e mating success) of the female moths decreased significantly with advancing age so as the coefficient of egg laying and fertility. The mated females laid maximum number of eggs within first 24 h of depairing, but the egg laying of the virgin females were found to be slow, erratic and delayed. The survivality of the moths during oviposition significantly decreased with the advancing age in both mated and virgin females.

14-026

A MODEL FOR A COMMON INTEGRATION MECHANISM OF THE RETROTRANSPOSON , BMC1, AND RETROPOSON, BM2, OF *BOMBYX MORI*

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We isolated a retrotransposon, BMC1, which was a member of the LINE family without LTR and is present in about 3500 copies per haploid genome. This element is considered to be integrated into the genome through an RNA intermediate without including any ORF for reverse transcriptase. Short SINE-like repetitive elements designated as Bm2 (~10⁴ copies) are homologous to the 3' end of BMC1. One of three types of Bm2 element was identical to the 3' end of BMC1 and hence both elements may be derived from the same ancestor. The same member (3.7kb) containing the complete ORF for reverse transcriptase was reported in the intron of amylase gene. This suggests that an active reverse transcriptase and integrase complex recognizes and integrates defective RNA intermediates into the genome. If this integration complex recognizes the 3' end of the corresponding RNAs, the same mechanism could occur in the integration of both elements. We will talk about the possible evolutionary origin and model for integration of both elements. Ogura,T.et al., Chromosoma 103, 311 (1994)

14-025

A HIGH-EFFICIENCY, NON-LYTIC EXPRESSION SYSTEM FOR PRODUCTION OF RECOMBINANT PROTEINS IN INSECT CELLS

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This expression system is based on the utilization of the cytoplasmic actin promoter of the silkworm *Bombyx mori*, which is constitutively expressed in virtually all cell types, including all available silkworm cell lines and many cell lines derived from other lepidopteran insects. The actin promoter is coupled to two genetic elements of BmNPV that enhance dramatically its activity and the expression of any genes linked to it. The first element is an enhancer that stimulates the actin promoter in *cis*. The second one is a gene encoding a transcriptional regulator. This stimulates the expression of the actin promoter in *trans*. Each of the two genetic elements increases the level of expression of genes cloned in the actin promoter-based expression cassette by approximately two orders of magnitude relative to that obtained from the basic actin expression cassette. Supplementation of the actin promoter with both genetic elements results in an increase of 2,000 to 5,000-fold in the transcriptional activity of the actin promoter and in a corresponding increase in the expression of recombinant proteins encoded by genes cloned under its control. A comparable degree of enhancement in the activity of the silkworm actin promoter occurs also in cell lines derived from other lepidopteran species.

When coupled with a hygromycin-resistance selection scheme allowing stable chromosomal integration of the recombinant expression cassettes, and with a cell cloning procedure resulting in the acquisition of cells expressing uniformly high levels of the recombinant proteins, this system yields quantities of recombinant proteins comparable to those achieved through conventional infection of insect cells by baculovirus expression vectors. To avoid cell poisoning caused by accumulation of large quantities of foreign proteins that are not normally secreted out of the expressing cells, we have also constructed a modified expression cassette containing a synthetic signal peptide sequence that directs recombinant protein secretion.

This non-lytic expression system has several advantages over baculovirus-based expression systems, including the capacity for production of recombinant proteins from both cDNA and intron-containing genomic sequences. These advantage will be discussed.

14-027

XANTHINE DEHYDROGENASE GENES IN THE OILY MUTANTS OF SILKWORM, *BOMBYX MORI*

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In order to develop a transgenesis in the silkworm, construction of suitable vector contained dominant marker gene would be very useful. In the silkworm, it is well known that many different morphological mutants are maintained. Among them, three different oily mutants located on *og* and *oq* loci are caused by low level or deficiency of xanthine dehydrogenase (XDH) activity. The mutant skin becomes translucent because no uric acid globules are formed in the epidermal cells. We recently succeeded to clone two XDHcDNA in the silkworm. The sequence of two genes shows reasonable similarity with other organism's XDH genes. Therefore, we call them BmXDH1 and BmXDH2. In this report, we analyzed the structure of two genes in the oily mutants by southern blotting. The comparison between the normal and mutant from *og* strain showed no significant difference. However, mutant specific BmXDH1 and BmXDH2 bands were detected in *oq* strain. We are now analyzing the meaning of these mutant specific bands and the low level of enzyme activity by using long PCR.

14-028

INTEGRATED GENETIC MAP OF THE *BOMBYX MORI* GENOME
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We constructed a linkage map of random amplified polymorphic DNAs (RAPDs) in *Bombyx mori* consisting of 168 loci on 29 chromosomes which we are integrating with a linkage map of restriction fragment length polymorphisms (RFLPs) by another group. We are also overlapping these molecular maps with the conventional linkage map for phenotypic loci, which requires large scale crossing experiments. The correlation of 14 chromosomes between the phenotype linkage map and our RAPD map is already known. For chromosomes 13 and 22, we have made integrated maps of a single line which carries both molecular and phenotypic loci. Our final goal is to find DNA markers that are closely-linked to phenotypic loci of interest, i. e., disease resistance, diapause, embryogenesis, behavior and so on, and to obtain the genes by positional cloning. Toward this end, we are determining the nucleotide sequences of RAPDs, and are designing specific primers to amplify the loci they represent.

We are also trying to map genes whose function and nucleotide sequence are known. We have mapped the gene encoding prothoracicotropic hormone at 2.5 cM on chromosome 22 and the gene encoding the diapause hormone and the pheromone biosynthesis activation neuropeptide at -2.2 cM on chromosome 11. We have found a polymorphism of the gene coding for the ecdysone receptor, and are looking for the chromosome on which it is located. The aim of these efforts is to know the relationship between growth-regulating molecules and already localized phenotypic mutants.

14-030

STRUCTURE AND FUNCTION OF THE SILK FIBROIN MOLECULAR COMPLEX
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Western blot analysis of the silk fibroin secreted into the lumen of the posterior silk gland or in the cocoon of *Bombyx mori* with polyclonal antibodies against synthetic peptides corresponding to segments in the fibroin L-chain and P25 or against the whole L-chain has demonstrated that L-chain is a major and P25 is a minor small protein components in the fibroin molecular complex. Combined approaches with gene cloning and peptide analysis have demonstrated that Cys190 in L-chain and Cys 20 (from the C terminus) of H-chain form a disulfide bond. The homozygous *Nd-sD* mutation, mapped to the *Fib-L* locus on the 14th chromosome, causes severe deficiency of secretion of fibroin and the naked pupa phenotype. Studies on this mutation by cloning and sequencing of the mutant L-chain cDNA and its gene reveal that the H-L disulfide linkage can not be formed because the mutant L-chain has a completely different C-terminal half sequence created by intronic recombination and exon shuffling in its gene. These results suggest strongly that the disulfide linkage of H and L-chains is essential for the efficient secretion of fibroin. On the other hand, P25 is not covalently bound to H-chain. Its association with either H or L-chain is suggested to be through hydrophobic interactions. P25 is characterized by extensive intramolecular disulfide linkages and glycosylation. Electrophoretic mobility of P25 as revealed by the reaction with Con A is altered in the *Nd-sD* mutant silkworm, suggesting that the state of glycosylation of P25 is affected when the H-L linkage is not formed. Presence of homologues of L-chain and P25 in distantly related fibroin-producing insects implies that formation of the molecular complex as shown in *B. mori* is a common strategy for producing a large amount of fibroin among different species of insects.

14-029

TRANSOVARIAN TRANSMISSION OF A FOREIGN GENE IN THE SILKWORM, *BOMBYX MORI*, BY *AUTOGRAPHA CALIFORNICA* NUCLEAR POLYHEDROSIS VIRUS
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When the silkworm *Bombyx mori* cell line, BmN cell, was inoculated with *Autographa californica* nuclear polyhedrosis virus (AcNPV), the increase of progeny viruses in the culture medium was observed. The silkworm survives inoculation with AcNPV, however, pupal-adult metamorphosis of AcNPV-infected pupae was arrested and the metamorphosis was induced by administration of ecdysteroid hormone. The titer of progeny viruses in the hemolymph of AcNPV-infected pupae was 7.3×10^5 PFU/ml at 20 days p.i. We introduced a firefly luciferase gene, expressed under control of *Drosophila* heat shock protein gene or AcNPV immediate early gene promoter, into AcNPV DNA. When the 5th instar larvae of the silkworm were inoculated with the recombinant virus, luciferase activities were detected in the virus-infected larvae and pupae (F0), and in the newly hatched larvae of the next generation (F1). The silkworm larvae were reared on an artificial diet and successively mated. The genomic DNA was extracted from 5th instar larvae of each generation (F0 to F3). A polymerase chain reaction (PCR) was carried out using 5' and 3' primers derived from the luciferase gene. The PCR amplification and Southern blot hybridization analysis demonstrated that the luciferase gene was transmitted through at least the F3 generation. The luminescence in the hemocytes from the 5th instar larvae of the F0 to F3 generation were also detected. These results show that AcNPV can be utilized as vector for the transovarian transmission of foreign genes in the silkworm.

14-031

EXPRESSION OF FOREIGN GENES IN THE SILKWORM *BOMBYX MORI* USING BACULOVIRUS VECTORS
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The nuclear polyhedrosis viruses (NPVs), comprise a genus within the Baculoviridae. Due to numerous advantageous characteristics as vectors, NPVs have been widely used for the expression of foreign genes using insect cell cultures. Whole insects (e.g. silkworm larvae using the *Bombyx mori* NPV (BmNPV) vector) have also been used to produce large quantities of specific polypeptides. For secreted polypeptides, the expression rate (mg of protein per ml of medium or hemolymph) in silkworm larvae is often over 100-fold higher (e.g. 1 mg mouse IL-3 compared to a few ug in cell culture) than that in in vitro culture. Post-translational modifications (e.g. C-terminus amidation) which occur in mammalian cells also occur normally in larvae and the expressed product can be purified by traditional biochemical procedures. An improved strategy for the collection of hemolymph from silkworm larvae and commercial-scale production of feline interferon alpha for use as a veterinary medicine will be discussed.

14-032

POTENTIALITIES OF THE DENSOVIRUS-DERIVED EXPRESSION VECTORS FOR INSECT TRANSGENESIS

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By inserting different reporter or selection genes (*lacZ*, CAT, luciferase, *neo^R*) in the cloned sequence of the *Junonia coenia* densovirus (*JcDNV*) genome, we succeeded in expressing these genes under the control of the promoter of either the structural (S) or the non structural (NS) viral genes in Lepidopteran cell lines. An unexpected result of these vectors was the observation that the recombinant viral genome could maintain in cells from generation to generation, leading to the stable constitutive expression of the reporter gene. Analysis of the DNA extracted from clones of transformed cells revealed that the recombinant *JcDNV* sequence could integrate into the cell DNA if the gene coding for the three NS polypeptides (NS1, NS2 and NS3) is functional. If this gene is deleted (Δ NS vectors), the complete plasmidic sequence containing the recombinant genome is maintained as an episome. We recently observed that the deletion of the sequence coding for NS3 resulted in a 10 fold increase of cell transformation efficiency which reached 10^{-3} - 10^{-4} . Thus, the *JcDNV*-derived expression vectors appear as good candidates for gene transfer in insects. To further investigate their potentialities in this domain, we micro-injected eggs of *Drosophila melanogaster*, *Ceratitis capitata* and *Spodoptera littoralis* with pBRJLacZ Δ NS3 plasmidic DNA, a construct expressing the *lacZ* coding sequence fused to the coding sequence of structural polypeptides. The results of expression of β -gal through larval stages of these insects will be presented.

14-034

MORPHOGENESIS OF THE PUPAL WINGS IN BOMBYX MORI: THE LEVEL OF A TRANSCRIPT ENCODING A LONG COLLAGENOUS DOMAIN IS REGULATED THROUGH CHANGES IN ITS STABILITY ?

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Morphogenesis of the pupal wings of *Bombyx mori* is triggered during spinning of the cocoon by a sustained peak of 20-hydroxyecdysone which elapses on 4 days. Analysis of the developmental patterns of some markers suggests that, contrary to what occurs in *Drosophila*, the differentiation of the pupal wing starts probably during the last larval molts.

One of these markers, *BmColl*, encodes for a protein of the collagen super-family and the structure of the known region corresponds to that of a non-fibrillar collagen. No strict homology with a known collagen can be established and the C-terminal domain of this protein has no counterpart in libraries. In wing discs as in epidermis the transcript level is closely correlated to that of ecdysone. During the larval life, the accumulation in response to the hormone is rapid, dose-dependent, and can be obtained in vitro in absence of protein synthesis. Conversely, no *BmColl* transcript is detected in pupal wings, whatever the in vivo hormone level. Both the structure of the 3' end of the transcript and its accumulation following protein synthesis inhibition alone suggest that this accumulation might result, at least for part, from the stabilization of the messenger controlled by the hormonal status. A model is presented. Ecdysone control of development mediated by changes in mRNA stability might be more important than generally estimated.

14-033

TRANSCRIPTIONAL TUNING OF THE GENE ENCODING THE SILK PROTEIN P25 IN THE SILK GLAND OF BOMBYX MORI

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In the silk gland of *Bombyx*, the genes encoding silk proteins are expressed in either the posterior or the median cells.

To understand the mechanisms controlling regional specialization of transcription, we examined the promoter of P25, a posterior cell-expressed gene encoding a fibroin-associated polypeptide. The spatial specificity of expression could be reproduced on extrachromosomal copies of P25-LacZ fusion genes by *in vivo* functional assays using biolistic as means to transfect DNA into silk gland cells. The test of a series of mutated promoters showed that posterior cell-specific transcription strictly depends on the proximal SGFB cis-acting element, but not one other suspected upstream cis-elements.

Comparative *in vivo* DNaseI footprinting of P25 chromatin by LMPCR of posterior and median silk gland cells revealed a protection of the SGFB cis-element and of a Forkhead/CAAT-like cis-element at -40 in the active cells. Both approaches led to conclude that P25 transcriptional activation via SGFB, a silk gland specific factor present in both median and posterior cells, requires the action of another factor that governs posterior cell specificity. The two factors are under cloning at the moment.

14-035

OVEREXPRESSION OF BOMBYX MORI PROTHORACICOTROPIC HORMONE ACCELERATES THE SPEED OF KILLING OF A BOMBYX MORI NUCLEAR POLYHEDROSIS VIRUS MUTANT LACKING THE ECDYSTEROID UDP-GLUCOSYLTRANSFERASE GENE

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The speed of killing of host insects of *Autographa californica* nuclear polyhedrosis virus (AcNPV) is increased by deletion of the ecdysteroid UDP-glucosyltransferase (*egt*) gene from the AcNPV viral genome. However, when the prothoracicotrophic hormone (PTTH) gene of *Bombyx mori* is overexpressed in *Spodoptera frugiperda* larvae using both wild-type AcNPV and an AcNPV mutant that lacks a functional *egt* gene, any further increase in the speed of killing is not observed.

In this study, we prepared three BmNPV mutants, Bmegt⁻ (a mutant lacking the *egt* gene), BmPTTH (a mutant expressing the mature *B. mori* PTTH subunit under control of the polyhedrin promoter) and BmPTTHegt⁻ (a mutant lacking the *egt* gene and expressing the mature *B. mori* PTTH subunit), and compared their effects on the speed of the killing infected *B. mori* larvae.

When the 4th and 5th instar larvae were infected with wild-type BmNPV and these three mutants, Bmegt⁻ killed larvae more quickly than did wild-type BmNPV, as had been previously observed with AcNPV. BmPTTHegt⁻, however, killed much more quickly than did Bmegt⁻, although BmPTTH killed at the same speed as did wild-type BmNPV. In addition, 5th instar larvae infected with BmPTTHegt⁻ displayed wandering and spinning behaviors much earlier than those infected with the other mutant viruses.

The results indicated that the effects of overexpressed *B. mori* PTTH on the speed of killing were suppressed when the *egt* gene was functional and that the differences between results obtained in *B. mori* larvae and in *S. frugiperda* larvae probably reflected the species-specific functions of *B. mori* PTTH.

14-036

DEVELOPMENT OF NEW METHOD FOR ARTIFICIAL INSEMINATION OF THE SILKWORM, *BOMBYX MORI*

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Little has been known about the artificial insemination in Lepidoptera including the silkworm since the reports by Omura(1936). Recently, we succeeded in the artificial insemination of the silkworm using a special instrument developed by Kanda & Tamura(1991). The instrument consists of capillary tube , gas-cylinder, pressure regulator, magnetic valve and foot-switch. The sperm was collected from vesicula seminalis of newly emerged male moth or from bursa copulatrix of female moth immediately after natural copulation. The each 4-6 μ l of sperm suspension with extract of glandula prostatica or of sperm from b.copulatrix were injected into b.copulatrix of newly emerged virgin female moth. The inseminated female deposited normally fertilized eggs just as that of natural copulation. Number of eggs laid and fertilized, and fertility were not different those from natural copulation significantly. The addition of the extract of g.prostatica was essential for both activities of sperm motility and fertilization.

The hatchability of eggs from artificial insemination was satisfactorily as high as that from natural copulation . The similar results to the sperm from v.seminalis were obtained when the sperm from b.copulatrix was injected to female moth. It is also confirmed by genetical method that fertilization by insemination was not caused by parthenogenesis but by sexual reproduction. Although the fertility was still low, the fertilized eggs were obtained from female inseminated artificially with semen which had been stored in liquid nitrogen with cryoprotectant.

14-038

IMPROVEMENT OF ARTIFICIAL DIETS FOR THE SILKWORM REARING ON ARTIFICIAL DIETS THROUGHOUT THE WHOLE INSTARS

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It was generally considered that artificial diets could not be used for silkworm rearing throughout the whole instars for silk production, due to the cost of the diets. With the development of low-cost artificial diets using a linear programming method and breeding of polyphagous silkworm races with a high adaptability to the diets this practice may become possible. However, low productivity of cocoon has been pointed out as one of problems to be solved in the silkworm rearing on artificial diets throughout the whole instars.

We found that the efficiency of conversion of ingested diets into the cocoon shell is lower in the absence of mulberry leaf powder in the artificial diets than in the presence. Moreover, the effect of dried grass powder, such as orchardgrass, added in the diets on the growth, cocoon quality and dietary efficiency of the silkworm was found to be almost the same as that of mulberry leaf powder, indicating that grass powder can be used for the feed ingredient instead of mulberry leaf powder.

Subsequently, we found that the growth of the larvae and cocoon quality are closely related to water content of the artificial diets. Cocoon quality, especially the weight of cocoon shell and the percentage of cocoon shell lowered with the increase in the water content of the diet. The suitable water content ranged from 60 to 65%, although it was found to be somewhat different between the two silkworm races, "Shin-Asagiri" and "Habataki"

On the basis of the above results, we developed a new type of artificial diet for the grown (5th-instar) larvae.

14-037

EFFECT OF THYROXINE ON THE GROWTH OF THE SILKGlands AND COCOON QUALITY IN THE SILKWORM, *BOMBYX MORI*

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The effects of feeding selected mulberry leaves treated with thyroxine on the growth of the silk glands and cocoon quality in the silkworm, *Bombyx mori* were analyzed. The results show that thyroxine greatly enhanced the growth of the middle division of the silk glands. Thyroxine treatment during the fifth instar larvae resulted in the highest quality of the cocoon filament.

14-039

METABOLISM AND UTILIZATION OF AMMONIA IN THE SILKWORM, *BOMBYX MORI*

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Silk production is the most important character in the nutritional physiology of the silkworm, *Bombyx mori*. There has been great interest in nitrogen metabolism and utilization in the silkworm in connection with silk production. The present study was aimed at determining if ammonia , a principal waste of nitrogen metabolism, could be utilized for silk protein synthesis and excreted as cocoon filaments by the silkworm. When diammonium citrate was supplemented in a diet containing only essential amino acids as nitrogen sources, growth of 5th instar larvae fed the supplemented diet was improved. The supplement produced marked effect on the growth of silk glands and resulted in a remarkable increase of several non-essential amino acids as glycine, alanine, serine, glutamine in larval hemolymph. By a tracer experiment using ¹⁵N ammonium acetate, utilization of a considerable amount of ammonia for silk production was revealed.

Next, presence of the pathway of ammonia assimilation in the silkworm was examined. When methionine sulfoximine (MS), a specific inhibitor of glutamine synthetase in plants, was injected in matured larvae of silkworm, ammonia concentration in hemolymph rapidly increased. On the contrary, glutamine concentration decreased. Incorporation of ¹⁵N ammonia into larval tissue proteins, especially silk protein, was strongly inhibited in the presence of MS. Spinning and development of larvae was also inhibited by MS. These results suggest that ammonia may be assimilated into amino acids in the silkworm via GS-GOGAT pathway as in plants or bacteria and finally voided as cocoon filaments.

14-040

PRACTICAL APPLICATION OF ARTIFICIAL DIETS FOR THE SILKWORM, *BOMBYX MORI*

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Artificial diets were applied for the first time in sericulture in Japan in 1977 for the rearing of young larvae of the silkworm, *Bombyx mori*, in cooperative rearing houses. Although, about 50% of the young silkworms are reared on artificial diets rather than on mulberry leaves in Japan, the cost of artificial diets accounts for about 50% of the total expenses for the silkworm rearing from the 1st to 3rd instars.

On the basis of the results of nutritional studies, low-cost artificial diets for the silkworm have been developed by applying a linear programming method to the formulation of the composition of artificial diets. In parallel with the development of the diets, studies on the feeding response of the silkworms to the diets and its inheritance have been carried out and polyphagous silkworm races with a high adaptability to the diets have been bred.

Development of a new type of silkworm rearing system based on these methods is deemed important.

14-041

AMINO ACID ABSORPTION IN THE SILKWORM MIDGUT
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Lepidopteran larval midgut absorbs amino acids through the coordinated activity of goblet cells and columnar cells. The former have a vacuolar-type H⁺-ATPase coupled to a K⁺/2H⁺ antiporter that generates a transepithelial voltage (DP) and a luminal K⁺ accumulation. In the latter the K⁺ electrochemical gradient is exploited by K⁺/amino acid symporters. These transport proteins operate in extreme chemical and physical condition, since pH value in the lumen reaches 12 units and DP across brush border membrane (BBM) exceeds 180 mV. We have measured the K⁺/leucine symport activity in BBM vesicles from *Bombyx mori* with varying pH and DP. The activity of the symport increases from pH 7.2 to pH 10.8, is poorly affected by intravesicular pH and is strongly activated by PD. Moreover alkaline pH also affects the efficiency of the transport, i.e. the accumulation of the amino acid. By fitting the data to a new implemented computer program that simulates the time course of amino acid uptake we evidenced that, in our experimental conditions, an important factor responsible for an increase in efficiency is the value of the initial uptake rate. Initial velocity increased more than 80% by passing from pH 7.2 to pH 10.8. The effect of external pH was qualitatively similar when analysing amino acid uptake in anterior, middle and posterior midgut. However, the symport is expressed unevenly along the midgut with the posterior region retaining the highest specific activity owing to higher efficiency and higher initial uptake rate. Several kinetic experiments support structural differences of the transport proteins along the midgut. The relevance of other factors able to modulate the symport activity in the silkworm (substrate specificity, hormones, and starvation) will be discussed.

14-042

UTILIZATION OF GRASS POWDER AS AN ARTIFICIAL DIET STUFF FOR SILKWORM (*BOMBYX MORI* L.)

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Utilization of some kinds of grass (ryegrass, Italian ryegrass, orchardgrass, alfalfa and white clover) was tried as a material of artificial diet for silkworm.

The nutrition value of the grasses used was analyzed for adjusting the diet composition in accordance with the nutritional requirement of silkworm. Mulberry leaf powder of a basal diet for silkworm was substituted by 10%, 20% or 30% of grass powder. Moisture content of the diets was ca. 74% and the pH in the range of 4.4 to 4.9, which were generally the same level as those of diet on the market.

Feeding the diets containing each of the grass powder from the 1st instar, the rearing results were superior to the feeding from the 2nd or 3rd instar, but the larval ingestion became deteriorated and the larval duration longer as the quantity of grass powder in the diet increased. On the other hand, the number of feces during the 5th instar was in negative correlation with the content of grass powder in the diet and in positive correlation with the cocoon quality.

In conclusion, the diet containing ryegrass or white clover was superior to those containing the other grasses in the larval ingestion. Therefore, the ryegrass and white clover powder were recommended to be utilized at the level of 10~20% in the diet as materials of artificial diet for silkworm.

14-043

DEVELOPMENT AND UTILIZATION OF ARTIFICIAL DIET FOR SILKWORM IN KOREA

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The earliest development of an artificial diet for silkworm was accomplished by a research team of KIST(Korea Institute of Science & Technology) in 1971. Since 1973, The cooperative rearing system for young silkworm with artificial diet has been studied in Sericultural Experiment Station(SES). Then, an automatic feeding machine was developed by KIST team and a new composition of artificial diet for silkworm was reported and named as SES-type diet by SES team.

In 1978, 4 varieties were selected as leading silkworm varieties suitable for artificial diet rearing and disseminated by SES. On the other hand, a mass application test of artificial diet in the farm was carried out with the scale of 150 boxes(silkworm eggs) in 1974, and the scale of the rearing with artificial diet has been increased year by year.

Besides, the present status, problems and outlook of the artificial diet rearing for silkworm in Korea will be presented and discussed.

14-044

SENSITIVITY OF FIBROIN IN SILK GLANDS OF *BOMBYX MORI* TO DIETARY PROTEIN LEVELS

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Fibroin is the major silk protein produced by the posterior silk gland and stored in the middle silk gland during the fifth instar development. Fibroin gene expression is known to be under the control of hormones and nutrition. The evidence for the nutritional regulation of silk gene expression is the increase in cocoon weight and shell weight when silkworm larvae are supplemented with nutrients like aminoacids, carbohydrates or proteins. The present study is aimed to identify the effect of dietary protein levels on the accumulation of fibroin in *Bombyx mori*. Feeding the chosen hydrolysed soy protein to silkworm larvae at the rate of 2mg/female larva and 4mg/male larva/day during the fifth instar development increased the total weight of silk glands. Parallel to this an increase in the levels of fibroin heavy (H) and light (L) chains were also observed on a 4 - 15% SDS-PAGE and the same was confirmed by densitometric scanning. The homology of the H and L chain polypeptides in control and supplemented groups was confirmed by Western Blotting. Studies pertaining to the levels of fibroin (H & L) mRNA in silk glands with respect to dietary protein are under progress.

14-045

REARING THE SILKWORM LARVAE ON A NEWLY DEVELOPED ARTIFICIAL DIET, PELLET DIET

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The new type of artificial diet for the silkworm, pellet diet, was developed. The new method for the preparation made possible to prepare the diet from the mixed powder in only one process of the twin-screw extruder under a high temperature and pressure for short time. This processing resulted in the formation of the pellet diet in which many babbles were contained. By dipping dry diet in an appropriate amount of water, the wet diet can be prepared instantly with ease. The basal composition of diet was designed by linear programming. Two kinds of diet in which the content of mulberry leaf powder was varied according to the silkworm strain, normal and polyphagous strains, were used. Using half-grown larvae reared on the wet diet during their young stages, the newly ecdysized 3rd or 4th-instar larvae were fed on the pellet diet. Then, the newly ecdysized 5th-instar larvae were continued to feed on the fresh mulberry leaves. The results were evaluated by comparing with those from the control in which the larvae were reared on the wet diet until the end of 4th instar by following on mulberry leaves in the 5th instar. The larval growth, survival and cocoon quality were not so much different between two groups. These results show that the pellet diet is practically available in the place of the usual wet diet, in particular for rearing the 3rd and 4th instar larvae. This diet is useful for making simple the diet processing and for labor saving in diet supply to the larvae.

14-046

STUDIES ON INTEGRATED PACKAGE FOR REARING OF TASAR SILKWORM, *ANTHRAEA MYLITTA* D.

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A B S T R A C T

Based on research findings at Central Tasar Research and Training Institute - Ranchi, an integrated package has been developed for rearing of tasar silkworm, *Antheraea mylitta* D. in order to harvest considerable yield with quality cocoons. The package includes - methods for maintenance of chawki garden and economic plantation, control of gall infestation, foliar spray of urea to increase leaf quality, brushing schedule, pre-requisite for rearing, incubation of eggs, young age rearing on chawki garden under nylon net, late age rearing techniques, method of harvesting and storage of cocoons.

Rearing with the integrated package has shown a significant gain with regard to Effective Rate of Rearing (16.52%), cocoons/Dfl. (22 cocoons i.e., 60.31%) and absolute silk content per Dfl. (146.47%) over conventional technique.

The paper also contains comparative economics of tasar silkworm rearing which proves the advantages of the package of improved techniques vis-a-vis conventional technique.

14-047

BIODIVERSITY AND BIOGEOGRAPHY OF SILKWORM RACES IN THE NORTH-EASTERN REGION OF INDIA.

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The North-Eastern Region of India is one of the most focal region of the world having maximum genetic diversity due to its congenial sub-tropical, eco-climatic and geographical position. The region has a distinctive position in the world sericultural map because of its commercial culture of all the four silkworm races namely Muga (*Antheraea assama* Westwood), Eri (*Philosamia ricini* Boisduval), Tasar (*Antheraea mylitta* Drury), and Mulberry (*Bombyx mori* L.). Among these, golden yellow muga silkworm is found nowhere in the world except in Assam, a state of the North-Eastern Region. It is noteworthy to emphasize that the Region is the homeland of all the wild counterparts of the domesticated forms of silkworms and their various host plants. Moreover, other wild silkworm races are also available in the Region. This has made this Region the most potential for sericultural development in the world.

The paper deals with the distribution pattern, intensity, collection, identification, rearing and evaluation of certain wild silkworm races and the domesticated forms. The findings of this study may help in breeding and conservation of silkworm races in the Region.

14-048

PRODUCTION OF THE SILKWORM (*BOMBYX MORI* L.) EGGS IN JIANGSU PROVINCEL. Li, Y. Z. Zhang¹, R. D. Lu¹

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Jiangsu province, the home of the silk, is one of the largest suppliers of the silkworm (*Bombyx mori* L.) eggs in China. At present, there are 41 silkworm seed farms among which include two first class basic farms to maintain the grand-parent and the great-grand-parent seeds (P2-P3) and six second class basic farms to produce parent (P1) eggs. Every year, these farms can release 20,000 disease-free-layings(dfls) of P2-P3, 2,800,000 dfls of P1 and around 6 million cases of the hybrid (F1) silkworm seeds. The qualified eggs produced in Jiangsu supply not only local farmers but also those of other provinces, and even they are exported to foreign countries.

To strengthen the silkworm seed production management, Jiangsu Silkworm Seed Corporation is authorized in the egg production plan, the quality control especially in inspection of *Nosema bombycis* and the price decision province-wide. Generally, the Corporation arranges the silkworm seed production plans for 41 grainages according to the orders given by the each county sericulture extension station which is in charge of surveying on the demands of the seeds, incubating the all eggs of whole county in the specific rooms, distributing the incubated eggs to the villagers and guiding them to rear silkworm as scientific methods.

Recent 40 years, the silkworm seed breeding has been made a great progress due to the scientific and technologic development and the silkworm varieties have changed for 4 times in a large scale in Jiangsu areas. The cocoon shell ratio has increased from 20% in 1950s to 25% at present, meanwhile, the cocoon filament length and the raw silk percentage of fresh cocoon has respectively increased from 1,000 m to 1,400 m and 14% to 20% for the spring season. It has been statistically showed that the major silkworm varieties extended in Jiangsu mainly bred from Sericultural Research Institute, Chinese Academy of Agricultural Sciences in Zhenjiang city.

14-050

POSTEMBRYONIC DIFFERENTIATION OF THE GONADS IN HONEY BEE (*APIS MELLIFERA* L.) QUEENS, WORKERS AND DRONES

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Sex- and caste-specific morphogenesis of the larval gonad is a prerequisite for the realization of different reproductive strategies in drones and queens on the one hand, and for reproductive division of labor between a fully fertile queen and nearly sterile workers on the other. As compared to ovaries, development of the larval testes is considerably advanced. Already in the fourth larval instar, somatic and germ line cells in the testis tubules become distinguishable, and spermatogonia clusters with fusome-like structures can be found. Spermatocytes enter meiosis concomitant with the pupal moult, and shortly thereafter spermatids initiate spermiogenesis.

In contrast, differentiation of somatic and germ line cells in the ovary only occurs at the beginning of the fifth larval instar. A critical aspect of germ cell differentiation, the formation of syncytial germ-line cell clusters, was found to occur only in queens. Such clusters connected by a central fusome are not formed in most of the larval ovarioles in the worker ovary, instead histological signs of programmed cell death can be found. Formation of the fusomes coincides with caste-specific differences in juvenile hormone titers. Application of synthetic juvenile hormone induced cluster formation and prevented cell death in worker ovarioles. Whereas juvenile hormone appears to play a role in ovary differentiation at the cellular level, ecdysteroids were shown to be involved in the regulation of protein synthesis in the larval ovary. Makisterone A specifically inhibited the synthesis of a 29 kD heat shock-inducible protein. N-terminal microsequencing of this protein revealed homologies to actin-binding proteins, in particular the *Drosophila* Hu-li-tai-shao protein. Caste-specific ovary morphogenesis, thus, appears to depend on the correct timing of corpora allata and prothoracic gland activity.

14-049

COMPARATIVE STUDY ON MICROSPORIDIAS PATHOGENIC TO *BOMBYX MORI* L. (LEPIDOPTERA : BOMBYCIDAE)

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Four kinds of microsporidian spores were isolated from silkworms and wild insects, i.e. MA-1 and MD-1 from silkworm rearing production, Pha-M from a mulberry insect *Phyllobrotica armata* in Sichuan province and Ha-M from mulberry looper, *Hemerophila arilineata*, in Jiangsu province.

These microsporidian spores were compared in the following aspects respectively with *Nosema bombycis*, the typical pebrine pathogen of the silkworm. The results are as follows: 1) Spores size -- The spores of *Nosema bombycis* is ovoidal and $3.59 \pm 0.16 \times 2.07 \pm 0.12 \mu\text{m}$ in size, and the spores of MA-1 ovoidal and $3.79 \pm 0.17 \times 2.05 \pm 0.13 \mu\text{m}$, the spores of MD-1 elongate-ovoid and $4.06 \pm 0.20 \times 1.66 \pm 0.10 \mu\text{m}$, the spores of Pha-M ovoidal and $5.05 \pm 0.18 \times 3.07 \pm 0.13 \mu\text{m}$. 2) Exospore and total proteins of the spores -- The exospore protein of *Nosema bombycis* consists of 3 subunits with the molecular weight of about 32000, 26000 and 24500 Daltons respectively. The SDS-PAGE profiles of total proteins of *Nosema bombycis* spores contain at least 25 bands. Difference in protein components was found among those microsporidian (*Nosema bombycis*, MA-1, MD-1, Pha-M, Ha-M). 3) Pathogenicity to the silkworm and serological relationships -- Among these microsporidias, the Pha-M shows poorest pathogenicity to the silkworm while MD-1 shows the strongest pathogenicity. Except the Pha-M, other three microsporidias showed serological reaction with the antibody of *Nosema bombycis* spores. 4) Parasitizing site, pathological changes in infected tissues and cells and life cycle in the silkworm. Those microsporidias mentioned above can parasitize most tissues and organs in the silkworm. The cells parasitized with the microsporidias showed greatly hypertrophied, the nucleus smaller or squeezed to the edge of the cells, the cytoplasm not dyed by the Eosin because of cells full of the spores. The life cycles of the MA-1, MD-1 and Ha-M are similar to that of *Nosema bombycis* in the silkworm besides the spore formation of Pha-M was delayed slightly.

14-051

RACING FOR A QUEEN:

DRONE MATING FLIGHT AND MALE COMPETITION

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Drones of the honeybee (*Apis mellifera* L.) perform mating flights under conditions of strong male competition. Often queens are pursued by large groups of drones, which persist behind her for some time. The subject of our study was the competitive behaviour of drones. A tethered queen dummy and stereoscopic video equipment were installed on a natural drone congregation area. The dummy was moved with constant speed on a circular trajectory. Drones were able to follow, mount and even copulate with the queen dummy. The size of the observed groups varied frequently. The behaviour of the drone during his mating flight seems to be influenced by the group of competitors. Singly approaching drones perform a chasing flight. They tend to fly almost straight to their target. In contrast, members of groups show complicated interactions, while competing for a better position in the drone cloud behind the queen. The shape, size and orientation of the cloud are fairly stable, but the internal drone constellation changes continuously. Drones are able to keep a certain minimal distance to each other. Nevertheless, occasional bumping can be observed too. Drones show a mating strategy which is split into two behavioural patterns, either chase or persistence. The described behavioural dichotomy reflects the rapid changes of mating situations under natural conditions. Reaching a freely flying queen requires a straight chasing flight. But while the queen is still engaged in copulation, a drone must keep his position for a next attempt.

14-052

REPRODUCTIVE ISOLATION AMONG SYMPATRIC ASIAN HONEYBEES

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The genus *Apis* (A.) is a 'good' systematic unit and its synapomorphic characters concern many aspects of morphology, physiology and behaviour. Similarities in sex pheromones and mating behaviour cause deteriorating interactions which interfere with natural mating of the 'minority' species wherever the Western *A. mellifera* and the Eastern *A. cerana* are brought together. But in case of sympatric Asian honeybees naturally 3 or even 4 species are found closely together in many regions. Experiments and observation on reproductive isolation were undertaken in the area of Anuradhapura (North Central Province, Sri Lanka) with 3 sympatric species (*A. florea*, *A. dorsata*, *A. cerana*) and in Tenom (Sabah, Malaysia) with *A. dorsata*, *A. cerana*, *A. koschevnikovi* and *A. andreniformis*. In both locations the seasonal timing of mating was similar in all species: drone and queen production was synchronised and started parallel to a significant seasonal increase in availability of pollen and nectar. In Anuradhapura and Tenom times of daily mating flights were species specific, allowing each bee species its own, well separated time frame for mating. In *A. cerana* this time frame differed regionally and the median time for mating flights ranges from 12.00 (North Pakistan) to 16.30 (Sri Lanka). In *A. dorsata*, however, the mating time was similar in each place (on the basis of very few records only) and mating flights occur during a short period in the dusk of the evening. Differences in mating time form an effective 'pre-mating barrier' and are the main cause for reproductive isolation among sympatric *A.* species.

14-054

CURRENT STATUS AND EMERGING TECHNOLOGIES OF HONEY BEE REPRODUCTION

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Molecular studies at the Beltsville Bee Research Laboratory (BBRL) have shown that there is significantly less genetic diversity in the U.S. commercial honey bee breeding population than in feral and old world populations. DNA-RFLP and PCR studies on sperms of individual drones are being used to determine the nature and extent of variability as well as to provide diagnostic markers for subspecies and populations.

DNA markers can be used for semen identification and certification. Artificial insemination of queens by using selected semen type would provide new ways of bee breeding. Commercial beekeepers could get into their stocks desirable genetic traits and genetic variability. However, suitable sperm cryopreservation methods are lacking.

The semen preservation methods are needed to establish bee sperm banks. The ongoing research at BBRL is focused on the development of bee germplasm preservation technology. This includes understanding the mechanism by which the sperm in the spermathecae remain viable for 3-4 years. Use of organ culture method for spermathecae is being looked into. In addition, methods for cryopreservation of eggs and early zygotes, as well as for DNA analysis on eggs, are being investigated. Results of these and other studies will be presented.

14-053

MALE REPRODUCTIVE ORGANS AND MODE OF MATING IN THE GENUS *APIS*

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Mating in the genus *Apis*(A.) occurs in free flight and is further characterised by male monogamy and female multiple mating. Since the eversion of the huge endophallus leads to the paralysis of the drone, special mechanisms for coupling the partners are required to ensure sperm transfer.

In *A. mellifera* the coupling between the queen and the paralysed drone is achieved by the endophallus. In the stage of sperm transfer the endophallus is everted up to the cervix and the voluminous vestibulum contains the bulb with mating sign. After full eversion the drone falls to ground, leaving his sperm in the oviducts and the mating sign in the sting chamber of the flying queen. The mating sign is removed by the following drone prior to his own sperm transfer. After mating with about 10 drones the queen returns to the colony. About 5% of the spermatozoa collected in the oviducts will reach the spermatheca. Drones of the Asian cavity nesting honeybees (*A. cerana*, *A. koschevnikovi*) have an endophallus type similar to *A. mellifera* and these queens also collect a sperm number of at least about 10 drones in their oviducts during mating flight. In conclusion, mating and sperm transfer in all cavity nesting *A.* species seem to follow a similar pattern.

The drone's reproductive organs of the free nesting species are different. The dwarf honeybee drone does not produce mucus. The copulatory connection to the queen is attained by a special construction of the drone's hind leg. Queens returning from mating flight have no spermatozoa in their oviducts but only in the spermatheca. Thus sperm is apparently directly transferred into (the sperm duct of) the spermatheca by drones.

In *A. dorsata* microsatellite DNA analysis showed that queens are mated by an average of 30 drones. The sperm number per drone (2.5 mio) and the sperms in the spermatheca of queens (6 mio) seem to indicate sperm collection during mating in the oviducts. Further, the production of mucus may point to a type of sperm transfer in *A. dorsata* similar to the hive nesting bees. Nevertheless the *A. dorsata* endophallus with extremely long cornua and an elongated cervix has an unique morphology and its function during copulation is not yet understood.

14-055

REPRODUCTIVE COMPETITION IN THE CAPE HONEYBEE (*APIS MELLIFERA CAPENSIS*)

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Reproductive dominance is usually linked to morphological caste in honeybees, *Apis mellifera*, with the queen as the reproductive and the workers as the infertile individuals. However, in queenless colonies of the Cape honeybee (*A. m. capensis*) workers can establish themselves as pseudoqueens expressing all features of reproductive physiology which are otherwise typical to the morphological queen caste. Within a few days after queen loss *A. m. capensis* workers can develop to a fully functional pseudoqueen producing female offspring through thelytokous parthenogenesis. Only very few workers produce offspring and the majority of the workers remains sterile. Since the queen is highly polyandrous and many subfamilies of supersisters coexist in the colony, there is a dramatic change in the genotypic composition of the colony after pseudoqueen take over. Most subfamilies disappear from the colony. The pseudoqueen determination appears to be under genetic control. Workers of the specific dominant subfamilies established themselves in independent trials as pseudoqueens, outcompeting workers of the other subfamilies. Strong intracolony genotype selection governs which workers are to become pseudoqueens and which remain sterile.

14-056

DRONE CONGREGATION AREAS AND MATING STATIONS FOR HONEY BEE QUEENS IN THE ALPINE MOUNTAINS

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For a breeding program of honeybee queens in the Alpine areas good mating stations in the mountains are necessary for controlled mating. The site of the drone congregation areas around the mating station is important for the quality of this mating station.

Experiments were carried out in a steep mountain valley between Mariazell and Wildalpen in Austria. In the centre of the area Cordovan (cd) drones were kept, while black drones were flying only on the periphery. The virgin cd queens were placed for open mating in four directions at different distances (0, 2, 4, 6, and 8 km) from the centre. In all mated queens the worker offspring were controlled in order to find out the mating distance and the flight direction of the queens.

In Kärnten (near Möllbrücke) on a steep slope in an altitude of 1600 m cd drones were located. Up to 2000 m (3,3 km from the valley = 750 m altitude) and down to 1300 m altitude (2,7 km from the valley) 6 cd queens each at 100 m altitude were located for open mating. Dark drones were flying only at 900 m or lower.

The results show that the physiography seems to have a major influence on the queen's choice of a mating place. A close similarity between the orientation of drones (shown by F and H RUTTNER) and queens were demonstrated in these experiments. The mating distance was at maximum 6 km when the „view“ between queens and drones was open and lower than 2 km when there was a mountain or a narrow gorge („covered view“) between drones and queens. The difference in the altitude has no influence on the mating distance; cd-queens located for the mating flight at 2000 m altitude were also mated with dark drones like the queens at 1300 m.

14-058

COMPARISON OF NATURALLY AND INSTRUMENTALLY INSEMINATED QUEENS OF THE ITALIAN HONEYBEE (*APIS MELLIFERA LIGUSTICA*).

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This research project set out to examine a number of anatomic and physiological traits related to the type of mating (naturally mated = NM; instrumentally mated = II) in 57 2-year old queen honey bees of ligustica race.

Comparison between the number of ovarioles from the two ovaries (right and left) did not highlight any significant differences.

Both the weight of the queens (NM: $x=219\pm24$ mg; II: $x=222\pm30$ mg) and the number of ovarioles (NM: $x=345\pm38$; II: $x=351\pm42$) were not significantly different for the two groups. Despite this, the relationship between weight and the number of ovarioles was a differentiating element between queen bees with different mating method. There was no correlation between these two parameters for the NM queens ($r=-0.04$ $P=0.82$) but in II queens the correlation is positive and significant ($r=0.49$ $P<0.01$). There was no proportion between the size of the ovaries - divided into 3 categories, and the number of ovarioles. As regards ovary size, comparison of the two groups highlighted a different frequency distribution with a valuable higher proportion of II queens ovaries in the average category (II: 73.8%; NM: 57.6%). II queens also showed more uniform ovariole development. Our research suggests that a different ovariole functional status, growth cycles and functional rhythms are normal condition in every queen. The amount of sperm in the spermatheca was significantly higher for the NM queens (NM: $x=2.62\pm0.88$ millions.; II: $x=1.82\pm1.06$ millions).

14-057

DRONE CONGREGATION AREAS AND TIME OF MATING FLIGHT OF INDIGENOUS *APIS CERANA JAPONICA* AND IMPORTED *APIS MELLIFERA*

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The difference in the mating flight time of sympatric *Apis* species is thought to be a key factor in interspecific reproductive isolation. However, there is no detailed information about the interaction between indigenous *Apis cerana japonica* (Acj) and imported *A. mellifera* (Am). We compared the mating behavior of both queens and drones of the two species in the same biotope in Japan. Queens of Acj leave their hives between 13:15 and 17:00 while Am queens depart between 12:15 and 15:00. Queens with mating sign return as late as 14:45 to 16:35 for Acj, compared to 13:00 to 14:40 for Am. Drones fly from 13:15 to 16:30 for Acj, and from 11:30 to 15:00 for Am. In observations in Tokyo, both departure and mating flight times of Acj are 1.5 to 2 h later than those of Am. An Am drone congregation areas (DCA) was found 300 to 750 m south of our university apiaries. It is an open space surrounded by low trees. On the other hand, Acj DCA was found at 10 m above the canopy of *Quercus acutissima* trees.

Mating behavior was observed using a circling pole. Mounting behavior by Am drones on Acj queens was observed, and one drone even copulated. Therefore, Acj queens can mate with Am drones if they met. Am queens were inseminated with mixed semen of Am and Acj drones. The number of emerged Am workers corresponds well with the concentration ratio of Am spermatozoa. Interspecific copulation, if it ever occurs, has a deleterious effect because interspecifically-fertilized eggs do not hatch. When Am was first introduced into mainland Japan, however, it was suggested that both species had been separated chronologically due to the different allopatrically-developed mating timing. In addition to this, the reproductive isolation in the two species in Japan has been achieved also by spatial difference of mating place.

14-059

TEMPERATURE IN HONEY BEE BROOD CELLS AND ITS IMPACT UPON REPRODUCTION OF THE HONEYBEE PARASITE *VARROA JACOBSONI* OUDEMANS

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The parasitic mite *Varroa jacobsoni* Oudemans can only reproduce in capped brood cells of honey bees (*Apis*). Female mites lay up to 6 eggs, in intervals of about 24 h. Goal of the present study was to determine whether host and parasite have different temperature optima for developmental rate.

I. In field: The average temperature in brood cells during tests conducted from 8:00 p.m. to 8:00 a.m. ranged from 32.0°C to 35.4°C. In average temperature in brood cells located within the center of the brood nest was 1.8°C higher compared to temperature in cells located at the periphery of the brood nest. Besides position within the brood nest also ambient temperature, number of bees in the colony, the size of the brood nest and the number of bees per number of brood cells have a significant impact upon the temperature within the brood cell.

The results of measurements conducted with colonies of different honeybee races indicated, that differential race specific colony development causes differences in brood temperature during certain periods.

II. In incubator: Within the temperature range from 32°C to 35°C a temperature difference of 1°C during the capped brood phase results in a difference in development period of the bee brood of up to 33 h. If brood cells are kept at temperatures below 34°C, in some cases the increased development period of the bee brood is sufficient for even the 3rd-5th mite egg to reach adulthood. The percentage of mites producing offspring was not found to be dependent of temperature. In artificially infested brood cells kept at 33°C up to 4 daughter mites per mother mite reached adulthood whereas in brood cells kept at 35°C maximally 2 daughter mites per mother mite reached adulthood.

14-060

EXCHANGE OF *VARROA JACOBSONI* BETWEEN COLONIES OF *APIS MELLIFERA* AND *A. CERANA* SHOWS THAT NON-REPRODUCTION IN WORKER CELLS IS A TRAIT OF THE MITE. Willem J. Boot, Johan N.M. Calis, Joop Beetsma, Dong Minh Hai¹, Nguyen Kim Lan¹, Tran Van Toan¹, Le Quang Trung¹, Nguyen Hung Minh²
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In colonies of European *A. mellifera*, *V. jacobsoni* reproduces both in drone and in worker cells. In colonies of its original host *A. cerana*, the mites invade both drone and worker brood cells, but after invasion into worker cells they do not reproduce. This phenomenon of non-reproduction in worker cells is probably crucial for resistance of *A. cerana* to *V. jacobsoni*, because the population can only grow during periods in which drones are reared. To test if non-reproduction in worker brood of *A. cerana* is due to a trait of mite or a trait of the worker brood, mites from bees in *A. mellifera* colonies were artificially introduced into *A. cerana* worker brood and vice versa. About 80% of the mites from *A. mellifera* colonies reproduced, in naturally infested cells as well as when introduced into worker cells of *A. mellifera* and *A. cerana*. Conversely, only 10% of the mites from *A. cerana* colonies reproduced, both in naturally infested worker cells of *A. cerana* and when introduced into worker cells of *A. mellifera*. Hence, the phenomenon of non-reproduction in worker cells is due to a trait of the mites. Our results have large implications for selection programmes to breed less susceptible bee strains. They show that non-reproduction in worker cells may only appear if the mite population has evolved by natural selection, which may be the case in African and Africanized bees. In other cases, fitness of mites that reproduce in worker cells has to be measured to identify honey bee strains in which there is natural selection for non-reproduction in worker cells.

14-062

VOLATILE CUTICULAR COMPOUNDS OF EUROPEAN AND AFRICANIZED HONEY BEES WITH RESPECT TO THE ATTRACTIVITY TO THE PARASITIC MITE *VARROA JACOBSONI*
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Varroa mites are able to distinguish between several age classes of larval and adult honey bees. Reproductive *Varroa jacobsoni* females only invade brood cells containing bee larvae in the last instar shortly before capping. Also during their phoretic phase on adult bees, the mites show a clear preference behavior for nurse bees. Volatile chemical substances of the host cuticle are thought to be involved in this specific host recognition behavior. We compared the chemical composition and the *Varroa*-attractivity of cuticular extracts of larval and adult developmental stages. As there are reports that bees and larvae of the Africanized honey bee in South America are less attractive to the parasite, we also compared host stages of European and Africanized honey bees collected at the same site. Samples were taken from Africanized honey bees and *Apis mellifera carnica* bees in Ribeirao Preto, Brazil and from *Apis mellifera carnica* bees in Germany. Comparative chemical analysis of extracts were carried out by gaschromatographic methods. The preference behavior of single *Varroa* mites was tested in a laboratory bioassay where either living host stages were offered or extracts were presented on dummies. By this tests we could confirm the preference of *Varroa* females for certain host stages and for the corresponding extracts. The gaschromatographic analysis revealed differences in the chemical spectrum of extracts obtained from different larvae and adult bees. Quantity and composition of certain compounds within the unpolar fraction seem to be responsible for the specific chemo-orientation of the *Varroa* females. Although there are existing some odor differences between European and Africanized bees, we hardly could discover any race-specific preference behavior of the mites.

14-061

TRAPPING *VARROA JACOBSONI* IN DRONE BROOD COMBS OF *APIS MELLIFERA* AS A BIOTECHNICAL CONTROL METHOD
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Varroa mites invade drone brood cells 12 times more frequent than worker brood cells. Therefore, trapping mites in drone brood is a useful biotechnical control method. Currently, trapping of mites in drone brood is applied in normal colonies. Therefore, a substantial part of the mites will escape by invading brood cells on other combs than the trapping comb. Trapping mites in drone brood will be much more effective when it is applied during periods when no other brood is present in the colony. Under these conditions only 462 drone cells are needed to trap 95% of the mites in a colony of 1 kg bees^{1&2}. This knowledge was used to design a method to trap mites in pairs of colonies. One colony of the pair was made broodless. The brood was given to the other colony. This colony was split in a broodless part with the queen and a part in which a new queen was reared and that became broodless when all the old brood had emerged. This provided three times the optimal opportunity for trapping mites in drone brood: a broodless (part of a) colony. The effectiveness of the method was 93%. Thus, trapping mites in drone brood is an effective non-chemical alternative to control *Varroa* mites in honeybee colonies.

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14-063

CONSEQUENCES OF CHANGING HOSTS ON THE REPRODUCTIVE BIOLOGY OF THE PARASITIC HONEYBEE MITE *VARROA JACOBSONI*
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Difficulty in following individual mites throughout their life under natural conditions has meant that basic factors such as mite longevity and fecundity have often been estimated by indirect methods. This has resulted in a wide range of values without a cohesive picture emerging. This makes developing mite population dynamic models very difficult. Recent field work has demonstrated that the average number of reproductive cycles carried out by the mite may be double that of previous field studies. This increase may account for the rapid build up of mites sometimes observed in the field and offers some explanation for the wide range of values obtained in past studies. Further modelling of these mite populations has raised questions that may be answered by looking at the differences between the two bee hosts. The change of hosts has placed the mite in a new reproductive environment. This may have resulted in an imbalance between its reproductive and biological age. This may account for some of the abnormal reproductive behaviours observed in its new host.

14-064

COMMENTS ON THE ANTIQUITY OF HONEY BEE SUBSPECIES (HYMENOPTERA: APIDAE, *APIS MELLIFERA*)

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The *Apis cerana*-*Apis mellifera* cladogenesis is estimated to have occurred about one million years ago, based on comparative morphological, physiological and behavioral data. Other species within the genus are believed to have diverged much earlier. Analyses of mitochondrial and nuclear DNA sequences collected from a number of *Apis* species, lead us to postulate a much more ancient time of divergence for the *A. cerana*-*A. mellifera* clade. However, when calibrated to a *Drosophila* rate model of sequence divergence, mitochondrial sequence differences among *Apis mellifera* subspecies support comparatively recent origins for the subspecies groups. The implications of these seemingly incongruous conclusions to models of subspecies formation and phylogeography in *A. mellifera* are discussed.

14-065

POPULATION STRUCTURE AND PHENOLOGICAL ISOLATION IN HONEYBEES: THE AFRICAN PERSPECTIVE.

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The honeybees of Africa have been characterized morphometrically as eleven distinct subspecies at the continental macrolevel. Multivariate analyses of features of low (morphometrics) and high (pheromonal) heritability at the microlevel yield clusters of distinct populations within races throughout Africa.

The potential for gene flow among honeybee populations in Africa is largely structured by climate and the phenology of flowering. These drive the seasonal cycles of honeybee colonies including build-up, swarming and migration. The sum of the effects of the forces operating on honeybee colonies and their responses to them result in differing degrees of probability for the isolation of populations making them temporally allopatric. Whether these populations are in fact subspeciating is moot.

14-066

TESTING HYPOTHESES FOR THE EVOLUTION OF POLYANDRY IN THE HONEYBEE (*APIS MELLIFERA* L.) WITH SINGLE-LOCUS DNA-FINGERPRINTING

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The honeybee queen mates with an extreme number of males, in some species exceeding 50 drones per queen. Here we use 40 *Apis mellifera* colonies, which were kept for performance evaluation, to test the different models for the evolution of polyandry (reviewed by Keller and Reeve 1994). The number of patrines of each colony was estimated with single-locus DNA-fingerprinting using four different loci. The number of effective matings per colony (ranging from 5 to 28.5) shows a positive correlation with the honey yield ($p < .05$). We found no evidence for a reduced susceptibility to parasites (tested organisms: *Varroa jacobsonii* and *Nosema apis*) as predicted by the theories of Hamilton (1987) and Schmidt-Hempel (1994). Moreover we found no increased variance for brood production in colonies with a lower number of patrines as predicted by Page (1980). Our results support the concept of increased colony fitness through genotypic variability capitalising on improved task specialization or greater homeotic capacity (Page and Robinson 1991, Crozier and Page 1985).

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14-067

A NEW POLYMORPHIC AND DIFFERENTIATED ENZYME LOCUS: PEPTIDASE-2 IN EUROPEAN *APIS MELLIFERA*

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Low levels of isozyme variation have been detected so far in European *Apis mellifera*. Three loci: malate dehydrogenase-1 (*Mdh-1*), esterase-3 (*Est-3*) and esterase-6 (*Est-6*) were successfully used in differentiating populations of different subspecies (Badino *et al.*, 1982, 1983, 1984, 1985; Bitondi and Mestriner, 1983; Marletto *et al.*, 1984; Sheppard & McPheron, 1986; Biasiolo & Comparini, 1990).

Data are presented here on peptidase-2 (leu-ala) locus. Its allele frequencies were compared among populations of *A. m. mellifera* (France and Corsica), *A. m. ligustica* (Piedmont, Latium, Calabria and Sardinia) and *A. m. sicula* (western and eastern Sicily). The *Pep-2¹⁰⁰* allele showed high frequencies (>0.90) in *A. m. ligustica* populations, whereas not reaching 0.30 in the *A. m. mellifera* samples, which showed a prevalence of *Pep-2¹¹⁰* (frequency 0.70-0.80). Population samples of *A. m. sicula* showed a distinct allele: *Pep-2¹⁰³*, at nearly equal frequencies with *Pep-2¹⁰⁰*. It is expected that this polymorphic and geographically differentiated locus will be useful, together with other molecular markers, to characterize different subspecies and populations of *A. mellifera* and to analyze hybridization and introgression phenomena.

14-068

MAPPING QUANTITATIVE TRAIT LOCI FOR HONEY BEE FORAGING BEHAVIOR

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Honey bees (*Apis mellifera* L.) forage for pollen and nectar and store surplus quantities in the nest. The amount of stored food materials is a result of behavioral decisions of workers to forage for pollen or nectar, the sizes of the loads they carry, the rates at which they forage, and the food consumption rate of the colony. We selected strains of bees for the amount of pollen they store. Within five generations, the high strain stored six times more pollen than the low strain. We then mapped two quantitative trait loci (QTL) that explained about 59% of the total phenotypic variance observed in a backcross population. These same two QTL were independently shown to have strong effects on the pollen and nectar load sizes of individual foragers.

14-070

THE IDENTIFICATION OF AFRICANIZED HONEY BEES: AN ASSESSMENT OF MORPHOLOGICAL, BIOCHEMICAL, AND MOLECULAR APPROACHES

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Africanized honey bees have spread through the Americas in the past 40 years. Desires to Monitor their progress, for survey and detection, regulation and research have lead to several identification approaches. Morphological procedures were developed in early studies of Africanized honey bees and have been improved to produce both rapid screening procedures for field use and laboratory procedures which are the officially authorized procedures of most countries and agencies throughout the world interested in identifying Africanized honey bees. Other approaches, especially biochemical and molecular ones, have use or promise as additional identification tools. Isozymes, mitochondrial DNA, RFLP, RAPID, microsatellite and minisatellite analyses of nuclear DNA, cuticular hydrocarbons, and non-specific proteins have all received experimental attention. Each approach has limitations in comparison to morphology although some have yet to be brought to their full potential.

14-069

POPULATION GENETIC STUDIES OF NATURAL HYBRIDIZATION AMONG AFRICAN HONEY BEES (HYMENOPTERA: APIDAE)

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In mountainous and savanna regions of East Africa, a clear separation between honey bees inhabiting the mountain forests, *Apis mellifera monticola*, and bees of the savanna, *A. m. scutellata*, is found. This discrimination can be observed morphometrically and regarding the allozymes and the mitochondrial DNA of 43 samples studied. Samples classified as *A. m. monticola* have different mitochondrial haplotypes from *A. m. scutellata*. Also, *monticola* bees show a greater variability in their morphology and a higher degree of heterozygosity in their allozymes, compared to bees from the savanna. These results are discussed as consequences of a refugial situation of the mountain bee populations descending from a large pleistocene population. In two of the three areas studied, there seems to be some, but limited, gene flow between mountain and savanna bees. At Mt Elgon and Mt Kenya, only a few samples were found with the morphology and allozymes of one race, but the mtDNA of the other one. However, the situation in the third area is different. The morphological discrimination between mountain and savanna bees collected in the Ngong Hills is less distinct and the allozymes of all the bees collected here appear to be similar to *A. m. scutellata*. These findings are confirmed by the fact that in all the Ngong samples only the mtDNA haplotype of *A. m. scutellata* is present. The hybridization of the two races in Ngong is discussed as a consequence of environmental changes in the region, mainly by deforestation and agricultural activities of the local human population.

14-071

THE ORIENTAL ORCHID LURES THE JAPANESE HONEYBEE WITH NASONOV PHEROMONE MIMICS.

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Flowers of the oriental orchid (*Cymbidium pumilum* Rolfe: Cp) attract workers, drones, queen and swarming colonies of the Japanese honeybee (*Apis cerana japonica* Rad.: Acj), but not of the European honeybee (*Apis mellifera* L.: Am). In this study, a hypothesis that the flower scent mimics the Nasonov pheromone of Acj was tested.

The scent of Cp flowers was analyzed by GC and GC/MS and found to comprise acetone, acetophenone, 2-heptanone, 2-nonanone, benzaldehyde, n-heptanal, n-octanal, n-nonanal, linalool oxide (cis, trans) of both furanoid and pyranoid, (S)-(+)-linalool, benzoic acid, fatty acids. When bioassayed by a polyethylene bag method, a real flower attracted 97% of workers and drones, whereas 0.1 flower-equivalent extracts attracted 96% of Acj workers and drones. Neither a real flower nor the extracts was attractive to Am workers and drones. As the Acj workers gathered toward the Cp scent, they exposed their Nasonov glands. Acj Ng extract attracted 98% of Acj workers and drones. We conducted a chemical comparison between the Cp flower scent and the Ng extract. GC profiles of Ng extracts from Am and Acj were quite different. Linalool, furanoid and pyranoid type of linalool oxide (cis, trans), fatty acids were detected in both the flower scent and the Acj extracts. Authentic (S)-(+)-linalool and linalool oxide (furanoid and pyranoid type, cis:trans=1:1) attracted Acj workers and drones at the 5fg to 500ng level, however the standard fatty acids mixture did not attract Acj in the filed and bag test. On the other hand, linalool and linalool oxide did not attract Am workers or drones.

In conclusion, the oriental orchid was found to have the same components in its scent as Acj had in its Nasonov gland, that is, linalool and four types of linalool oxide.

14-072

OBSERVATIONS ON THE ACTIVATION AND PATHOLOGY OF ACUTE PARALYSIS VIRUS IN THE HONEY BEE *APIS MELLIFERA* L. (HYMENOPTERA: APIDAE)

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Acute Paralysis Virus (APV) commonly persists as a latent infection in honey bees and is only known to cause mortality in nature in colonies infested with the parasitic mite *Varroa jacobsoni* Oudemans.

In initial experiments APV was induced to replicate to lethal levels in latently infected adult hosts by the injection of foreign proteins (e.g. haemocyanin) into the haemolymph. Apyrase (a component of tick saliva) acts in a similar way, suggesting that secretions introduced into the host haemolymph during mite feeding interfere with the host immune mechanisms which normally suppress APV replication.

The injection of young adult bees with lethal doses of APV, together with foreign proteins (mimicking mite vectoring), changes the dynamics of virus replication, both quantitatively and temporally.

14-074

RESEARCHES ON THE TREATMENT OF SOME VIROSES AND MYCOSES OF HONEY BEE *APIS MELLIFERA* L.

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In the last ten years very dangerous diseases spreaded all over the world, damaging the honey bees colonies. The acute paralysis of bees is produced by a virus, identified and isolated in laboratory from infected bees and then inoculated as a low pathogenic strain in honey bees colonies. The authors obtained the immunisation of bees to "Wild" virus attack. The honey bees mycosis are produced by fungi from *Ascosphaera* and *Aspergillus* genera and they are treated now with chemicals and antibiotics which lead to a slow intoxication of bees and to pollution of bees products. The authors found by testing several antifungal products, a better treatment, nontoxic and easy to apply.

14-073

EFFECT OF PERORALLY GIVEN BIOLOGICALLY ACTIVE SUBSTANCE OF ANIMAL ORIGIN ON MICROSPORIDIOSIS INFECTED HONEY BEES (*APIS MELLIFERA*)

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Biologically active substances (BASs) isolated from different animal systems and organs possess stimulative effect permitting to use them in medicine and veterinary. In this report we present some data concerning a BAS preparation, RBS, isolated from cattle embryo tissues and its effect on healthy and Microsporidia-infected (MI) honey bees (HB), both insects infected in laboratory and naturally infected having been used. In our laboratory experiments HBs were twice fed with 0.1 % RBS containing 50 % sucrose syrup (immediately after infection and 10 days post MI); in field experiments HBs were fed with RBS three times with 14 days intervals. In laboratory tests insects of the same age were infected in July-August (10,000 spores/HB); our field experiments were made with HB families with natural infection occurred in May-June. We found that RBS had no therapeutic effect in laboratory experiments. Furthermore, some MI stimulation took place accompanied by higher mortality of RBS-treated infected HBs comparing to untreated infected ones, the latter having smaller spores according to morphometric data. These facts are well correlated with a conception of obligatory parasitism postulating that factors favourable for a host organism are also favourable for its parasites, the disbalance developed in host-parasite system killing one of this system members.

The use of RBS in natural HB families with low MI frequency stimulated rather host than parasite organism, the treated families became stronger comparing to control ones. So it is necessary to be careful when treating parasites-carrying insects with BASs. Such preparations are to be used in apiculture only for HBs slightly infected or treated previously with antinosemic drugs.

14-075

IDENTIFICATION OF A HONEY BEE BROOD PHEROMONE

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Apis mellifera workers perform many necessary complex parental behaviors according to the age, the caste and needs of the brood. There is evidence for a brood pheromone indicating presence and state of the larvae in the cells. Since a blend of ten fatty acid esters had been identified on honey bee larvae, four of them was characterized as a brood pheromone triggering the capping of the cells by the workers. The blend of esters vary in quantity and in proportion of each of the compounds as a function of the brood age and caste. Behavioral tests, performed in natural conditions using larval and pupal lures including the compounds released in an amount similar to the amount naturally found on the larval cuticle, showed that modulation in quantities and in proportions of each ester as a function of age represents a chemical signature of old or young larvae for the workers. These compounds are also present on the queen pupal cuticle, and three of them are involved in the recognition and acceptance, by the workers, of queen cells containing a pupa. Some of these esters can also modulate the feeding behavior of the workers to the larvae. More, they can also act, in experimental conditions, on the development of the hypopharyngeal glands of the workers and are involved in the inhibition of the queenless worker ovaries. Thus, the blend of esters is a releaser and a primer brood pheromone which can be considered as important components in the chemical ecology regulating honey bee society.

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14-076

RADAR OBSERVATIONS AND REACTION TO QUEEN
PHEROMONE OF HONEY BEE DRONES (*APIS MELLIFERA* L.)
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Natural mating of honey bees occurs in the air, usually 10-25 m above ground sometimes at distances in excess of 3 km from the colony. Drones and queens both fly in the afternoon and drones in flight form flyways and drone congregation areas (DCA's). Our studies using a trailer-mounted X-band radar (9.4 gigahertz) documented the location of 18 km of flyways and 26 DCA's adjacent to a commercial apiary near Tucson, AZ.

The location of flyways was strongly influenced by local visual cues, especially tree-lines and roadways. The flyways were about 100 m wide (during maximum flight times) and were usually about 80 m to the side of tree-lines. The maximum height of drones in flyways was 21 m, whereas in DCA's they flew mainly from 30-50 m above ground (maximum height in a large DCA was 60 m). DCA location also appeared to be influenced by visual cues; abrupt changes in or termination of the visual cues seemed to induce the formation of DCA's. Some DCA's were consistently "bigger" either in area or numbers of drones than others.

Synthetic 9-ODA (queen substance) was used in the radar tests to confirm the presence of drones. In separate tests, other queen pheromone components (9-HDA, HOB, HVA) were tested at both 1 queen equivalent (Qeq) and at 10 Qeq and found to have no attractiveness to free-flying drones. However, drones responded positively when downwind 420 m from a mated queen and 800 m from a concentrated source of 9-ODA.

14-077

EFFECTS OF TWO PROTEINASE INHIBITORS ON DIGESTIVE
ENZYMES AND SURVIVAL OF HONEY BEES, *APIS MELLIFERA*
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Proteinase inhibitors have been shown to be potent plant defense factors against insects and their effectiveness when incorporated into transgenic plants to confer pest resistance has been demonstrated. Proposals to use such plants as part of an integrated pest management plan must take into account their effects on pollinator insects such as the honey bee.

Two endopeptidase inhibitors, BPTI (bovine pancreatic trypsin inhibitor) and SBTI (Kunitz soybean trypsin inhibitor) were found to significantly reduce the longevity of adult honey bees (*Apis mellifera* L.) fed the inhibitors *ad lib* in sugar syrup at 1.0%, 0.5% or 0.1%, but not at 0.01% or 0.001% (w:v). Bees were taken from frames at emergence, kept in cages at 33°C, and provided with a pollen/protein diet, water and syrup. *In vivo* activity levels of three midgut endopeptidases (trypsin, chymotrypsin and elastase) and the exopeptidase leucine aminopeptidase (LAP) were determined in bees fed either BPTI or SBTI at 1.0%, 0.3% or 0.1% (w:v) at two time points: the 8th day after emergence and when 75% of bees had died. LAP activity levels increased significantly in bees fed with either inhibitor at all concentrations. At day 8, bees fed BPTI at all concentrations had significantly reduced levels of trypsin, chymotrypsin and elastase. At the time of 75% mortality, bees fed BPTI at each concentration had reduced trypsin levels, but only those fed the inhibitor at the highest dose level had reduced chymotrypsin or elastase activity. Bees fed SBTI at all concentrations had lowered trypsin, chymotrypsin and elastase activities at both time points.

14-078

FLATLY SPUN COCOONS: PRODUCTION METHOD, CHARACTERISTICS AND ITS USE FOR HUMAN WELFARE

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A special silkworm race named 'Mayu-gami-ko' in Japanese meaning 'a silkworm race to produce flatly spun cocoons' was bred by the first author by crossing several silkworm varieties. Subsequent inbreeding for approximately seventy generations using an artificial diet as a sole diet fixed the race and its diet taste. By combining a silkworm rearing system on an artificial diet and aseptic rearing techniques, a new method to produce flatly spun cocoons on a plant scale was established that is free from anxieties such as unstableness of diet supply due to inappropriate weather or occurrence of insect disease.

Flatly spun cocoons usually take a form of a sheet like papers in sizes and shapes needed for various purposes and are composed of multiple layers of long silk threads attached together with natural glue protein called sericin. Its transparency makes the written figures on it look like floating in the air.

Flatly spun cocoons, now named 'San-ken-shi' in Japanese meaning 'a paper made of flatly spun cocoons of silkworms', can be used for the human welfare just like a Japanese paper; materials for calligraphy, paintings, arts, ornament, clothes, artificial leather, athletic goods, architectural interior, and medical science.

14-080

FT-IR STUDIES ON FIBROIN SECONDARY STRUCTURES OF WILD SILKWORMS

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The feasibility of silk fibroin for applications in high-performance fibers and biomaterial membranes depend largely on our knowledge of the structures of this natural product, which is critical for processing. FT-IR measurements of wild silkworm fibroin have been carried out to investigate fibroin structures. The curve-fitting analysis have been employed to estimate the secondary structure components with Fourier self-deconvolution procedures. These data showed that fibroin of *Samia cynthia ricini* have 45.5% helix, 17.0% sheet, 26.5% turn, and 11.0% disordered structures. Comparison of structures with other species will also be discussed.

14-079

THE MAIN TREND OF TUSSAH PRODUCTION OF LIAONING

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Tussah (*Antheraea pernyi*) is one of the peculiar biological resources of China, and is one of the economic insects which have great value in development. The tussah cocoon yield in Liaoning is 80% of that of China, and 70% of that of the world. It plays an important role in the tussah production of China and even of the whole world.

The main trend of tussah production in Liaoning will be: to pave a new way of comprehensive exploitation and multi-graded increment that means to develop silkworm farms fully and to process the tussah (including silkworm chrysalis, silk moths, silkworm eggs and larva) and silkworm excrement into biological products, such as insect made foodstuff, health products and biomedicines in order to increase benefits and serve the mankind; to pave a new way of intensive development with high-yield, high-quality and high-benefit that means to rise the labor productivity and the land output rate by improvement of tussah raising-tech, heighten of diseases prevention and curement ability, breeding a tussah of good strain; to pave a new way of ecological sericulture, that means to strengthen silkworm farms by improving its vegetation density and okra's canopy density in order to accomplish a better circle of ecological beneficial result on silkworm production.

14-081

STUDIES ON DOMESTICATION OF WILD TASAR SILKWORM
ANTHRAEA MYLITTA D - PERFORMANCE OF INDOOR REARED
YOUNG AGE SILKWORMS

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A B S T R A C T

In order to develop a method for indoor rearing of tropical tasar silkworm, *Antheraea mylitta* D., stagewise indoor rearing was conducted in wooden trays in room condition. Indoor rearing upto II stage followed by outdoor rearing on regular economic plantation of *Terminalia arjuna* has shown significant gain with regard to effective rate of rearing (13.43 - 18.16%) and absolute silk content per Dfl. (96-102.77%) over control (i.e., throughout outdoor rearing). The other economic characters of cocoons such as single cocoon weight, single shell weight and silk ratio were found to be at par with control. A decremental trend was observed in all the above parameters when rearing was conducted indoor for longer duration with minimum when reared upto cocooning.

The results indicate that indoor rearing may be conducted upto II stage and thereafter, larvae may be transferred to regular economic plantation to increase survivability and cocoon yield. Further studies with modifications are under progress to develop some appropriate technologies for domestication of tasar silkworm, *Antheraea mylitta* due its economic importance.

14-082

STUDIES ON ASSOCIATION BETWEEN COCOON WEIGHT AND SHELL WEIGHT OF MUGA SILKWORM (*ANTHRAEA ASSAMA* WESTWOOD) DURING DIFFERENT BROODS (LEPIDOPTERA : SATURNIIDAE)

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The extent of association between cocoon and shell of *A. assama* differed significantly during different seasons for both the sexes (male 'r'=+0.6035., female 'r'=+0.6887., df 48, both significant at 1 p. c level). The regression function of cocoon with shell was $Y = -0.02543 + 0.08212 X$ for male and $Y = -0.07756 + 0.07488 X$ for female during different broods. Both the variables of Jethua (May-June), Katiya (Oct-Nov) and Aherua (June-July) crops showed close and uniform affinity with the regression line and deviation recorded was maximum during Jarua (Dec-Feb). The factorial analysis of variance adduced the fact that association (A) of cocoons and shells was significant in both the sexes in all the broods. The interaction was significant at 0.1 p.c. level only in the case of female cocoons.

14-084

INSECT SPECIES WHICH ACCOMPLISH CROSS POLLINATION IN *RUBUS ULMIFOLIUS* SHOTT, 1818 (ROSACEAE)

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The reproductive strategies of *Rubus ulmifolius* Schott, 1818, which lead to allogamy, are based on a great range of factors which attract insects and lead to cross pollination. The morphological and structural of traits of the flowers are added to the offer of nectar and pollen allowing these rewards, to be available for a variety of insects.

The *Rubus ulmifolius* flowers received 1028 visitors (excluding *Apis mellifera* Linnaeus, 1758). They were selectively collected by an entomological net, during the years 1994 and 1995, in the flowering period of the plant. The localities are in Madrid (Spain): Casa de Campo (Madrid City) and Torremocha del Jarama (Northeast Madrid Province).

71 per cent of the collected insects are pollinators of this plant, and they belong to several families of the orders Hymenoptera, Coleoptera, Diptera and Lepidoptera.

The main pollinators of *Rubus ulmifolius* are bees (Hymenoptera, Apoidea), a 78 per cent of the total of the pollinators found, more than 45 species.

Wild bees are the most important group implied in the cross pollination of this plant, due to their special structural pollen-gathering features and their elevated rhythm of activity. This, besides their diversity and abundance.

14-083

Lac Z GENE EXPRESSION IN *BOMBYX MORI* EMBRYONIC TISSUES USING A DENSONUCLEOVIRUS.

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In an attempt to obtain germinal transgenesis in *B. mori* we examined integrative potentiality of Densonucleovirus of *Jujonia coenia* as a vector. The viral genome was cloned in pBR322 and the construct named pBRJ (1). A recombinant viral genome was obtained by inserting *lac Z* reporter gene in EcoRV unique site, under viral promoter control. This construct named pBRJlac Z allows easier expression studies (2).

We inoculated this vector in eggs 3 to 5 hours after laying near the micropyle and examined LacZ gene expression at different stages of embryonic development.

Expression of the reporter gene was observed in vitellophages for each experiment and in some time in tissues of two, four and ten day old embryos. For two day old embryos the nature of expressing tissues was not determined, but we observed, both in four and ten day old embryos expression in epidermal cells and in ten day old embryos expression in silk gland for one of them and in trachea for some of the others. However it was difficult to reproduce these results and in positive embryos the somatic expression frequency remained very low (<1%) and if it correlates the presence of viral genome, we can assume that it was too low for obtaining the statistical transgenesis of germinal cells. Currently we are studying the presence of viral sequences in genomic DNA of inoculated reared larvae by Southern blot.

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14-085

POLLINATION OF GREENHOUSE TOMATOES (*LYCOPERSICON ESCULENTUM* MILL.) (SOLANACEAE) WITH THE SOLITARY BEE *OSMIA CORNUTA* LATR. (HYMENOPTERA, MEGACHILIDAE).

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The possibilities of using the solitary bee *Osmia cornuta* Latr. as a pollinator of tomatoes (*Lycopersicon esculentum* Mill.) under confined environmental conditions was investigated.

The effect of the osmia-pollination was compared with natural self-pollination. The effectiveness of pollination was determined by measuring the percent of fruit-set on the first two trusses of the plants.

Fruit-set in osmia-pollinated plants resulted significantly higher (90,98%) than in the natural self-pollinated ones (50,6%). The *Osmia cornuta* did not increase its population but readily accepted to feed on artificial flowers filled with syrup and then visited the tomato flowers and pollinated them successfully. The analysis of the pollen provisions collected from the osmias artificial nests confirmed that the bees visit the tomato flowers and gather pollen from them.

These preliminary observations make the Megachilid bee *Osmia cornuta* an interesting alternative pronubial insect for pollination of tomato plants cultivated in confined environments.

14-086

WHEN FOREIGNERS MEET: BEHAVIOURAL INTERACTIONS BETWEEN AN ENDEMIC HOVER FLY (DIPTERA: SYRPHIDAE) AND INTRODUCED POLLINATORS (HYMENOPTERA) IN NEW ZEALAND
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Hover fly larvae can be important natural enemies of insect pests in many crops; the role of bees as pollinators is also of vital importance. In New Zealand, the most common hover fly is the native *Melanostoma fasciatum*; the native pollinator fauna is poor and honey and bumble bees have been introduced for this purpose. Both groups feed on pollen and nectar and interference may occur between them. It is important to devise management techniques that maximise the potential of both groups of organisms. The flowers of *Phacelia tanacetifolia* (Hydrophyllaceae) attract both groups. In this experiment, interactions between bees and hover flies on *Phacelia*, using both individual plants in pots and small plots, were studied. Techniques used included censusing of bees and hoverflies to obtain data on number of visits, visiting times, and flower choice; observations of the behaviour of individual insects, and comparisons of hoverfly behaviour in bee exclusion zones and control areas. Preliminary results suggest that although there may be some disturbance of feeding hoverflies by bees, weather conditions, especially temperature, and different diurnal activity patterns, are also of importance in dictating the peak foraging periods of each species. The implications of the results for management of field margins and flowering strips are discussed.

14-088

SEMIOCHEMICALS IN THE JAPANESE HONEYBEE, *Apis cerana japonica* Rad.
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In marked contrast to the European honeybee, *Apis mellifera* L. (Am), our knowledge on the chemical ecology of the Japanese honeybee, *Apis cerana japonica* Rad. (Acj) is limited. Therefore, comparative studies have done on the Nasonov gland, sting gland and head extract of Am and Acj by GC, GC/MS and by behavioral assays. In the Nasonov gland extract of Am, the presence of seven previously identified Nasonov pheromone was confirmed. On the other hand, Acj extract gave none of these compounds. Instead, linalool and linalool oxide (*cis* and *trans* of both furanoid and pyranoid) were identified. In a laboratory bioassay, Acj workers were attracted (52-94%) to authentic linalool oxide (5-500ng). Sting gland extract of both species gave isopentyl acetate and (Z)-11-eicosen-1-ol as major components. The absolute amounts of these two compounds found in Acj sting gland were lower than those in Am extract. Isopentyl acetate elicited alerting behavior in both species. 2-Heptanone was identified in head extract from both species. This compound is known to exist in mandibular gland of Am worker and is regarded as one of the alarm pheromone. The survey on the biological function(s) and the origin of 2-heptanone in Acj is now underway.

14-087

INFLUENCE OF GRASSLAND MANAGEMENT ON POLLINATING HYMENOPTERA.
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The effect of management practices on pollinating insects of grasslands (Wetzlar, Germany) was investigated by means of yellow, white and blue water traps. The sampling areas form a small scale pattern of differently managed orchards and meadows (sheep-pasture, moving-pasture, one-, two- and multiple-moving-meadow). The results of the ANOVA show that bees (Apoidea) and other aculeate apocritans reached highest density on sheep-pasture. For parasitic and gall-forming Hymenoptera, in contrast, no significant effect of management practices could be established. Significant taxon specific differences in the preference for colors could be demonstrated. Bees and other aculeate apocritans significantly preferred white traps. Bees were additionally strong attracted by blue traps. Parasitic and gall-forming Hymenoptera, in contrast, preferred yellow traps. This suggests that the exclusive use of yellow traps in many field studies on pollinating insects may be inadequate. In conclusion, our data show that Hymenoptera significantly respond to differences in management practices on a very small scale. This opens up new perspectives for the conservation of insect communities of grasslands.

14-089

THE HONEY BEE (*APIS MELLIFERA* L.) AS PESTICIDE BIOINDICATOR. EVALUATION OF THE DEGREE OF POLLUTION BY MEANS OF ENVIRONMENTAL HAZARD INDEXES
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The use of the honey bee as a biological indicator of pesticide pollution is mainly based upon its particular sensibility to most of the active ingredients used in agriculture. In fact, these insects react to the presence of pesticides in the environment showing various levels of mortality or bodily residues or a contamination of the hive products. The intensity of these effects depends upon the type of active ingredient used, the type of crops sprayed, the spraying time of day, the weather conditions during and after spraying and so on. A suitable innovative processing of the collected data can enable the drawing of detailed maps showing the degree of endangering of a certain environment, following similar assessed methods used in processing data coming from other bioindicators. In this frame we propose a data processing procedure that uses both toxicity informations for the pesticides detected on dead bees by means of chemical analysis and bee mortality values collected in the monitoring network stations, each one consisting of two hives equipped with cages for the collection of dead insects.

	no pesticide traces*	BEE MORTALITY CLASSES			
		0-100 D*	100-400 C*	400-800 B*	> 800 A*
LOW	0 - .25	D	D	C	C
MEDIUM LOW	.25 - .5	D	C	C	B
MEDIUM HIGH	.5 - .75	C	B	B	A
HIGH	.75 - 1.	B	B	A	A

The Table shows values of the Index of Environmental Hazard (IEH): A maximum hazard; B high hazard; C medium hazard; D low hazard. Results of IEH classes marked with * refer to situations where no pesticide traces have been detected in dead bees. Mortality classes have been set relying on the analysis of nearly 5000 weekly samplings carried out in Italy in recent years. The Index of Pesticide Toxicity (IPT) can be evaluated using the formula:

(IPT)_{month} = f_{corr} ∑_{c=1}^N (c̄t)_c (f̄p)_c / N

where (IPT)_{month} is the Index of Pesticide Toxicity over the month, (c̄t)_c defines the class of toxicity for sampling c normalized to the maximum expected value, (f̄p)_c is the persistency factor for sampling c normalized to the maximum expected value, f_{corr} is an (IPT) corrective factor over the monthly samplings and N is the total number of monthly samplings. The value of IPT is calculated using pesticide data available in literature. Crossing values of IPT and of averaged bee mortality leads to the obtainment of values for IEH, describing the level of pesticide pollution when referring to the sensibility towards it of the honey bees monitoring a certain environment.

14-090

LABORATORY AND OPEN FIELD INVESTIGATION OF *HERIADES TRUNCORUM* L. (HYMENOPTERA MEGACHILIDAE) DIET.

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A palinological investigation was carried out in central Italy, in the framework of bio-ethological studies on *Heriades truncorum* L., in order to study the favourite diet of these insects in the state environment, with the aim of tracing the pollen sources visited. Microscopical analysis of pollen gathered and stored in cells of artificial nests showed that although *Heriades truncorum* L. prefers flower of compositae; in the environment under examination the above insect can extend their diet to other species of other botanical families.

14-092

BACILLUS LARVAE PROTEINASE INTERFERES WITH ANTIBACTERIAL RESPONSE PEPTIDES OF THE HONEYBEE

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Insect has only a limited number of ways reacting to a considerable variety of bacterial pathogens. The honeybee defends itself against a wide range of bacterial invaders by an inducible immune system. Two classes of antibacterial immune proteins, the apidaecins (Casteels P. et al., EMBO J. 8, 2387-91; 1989) and abaecin (Casteels P. et al., Eur. J. Biochem. 187, 381-86; 1990) are well biochemically characterized inducible molecules in *Apis mellifera*. Some insect bacterial pathogens (Sidén I. et al., J. Gen. Microbiol. 114, 45-52; 1979) including *Bacillus larvae* (Jarosz J. & Gliński Z., J. Invert. Pathol. 56, 143-49; 1990), a causative agent of American foulbrood (AFB), produce exoproteases during parasitism that degrade inducible antibacterial immune proteins in holometabolous insects. As a result of depression of insect's non-self immune response, the pathogen develops in the haemolymph, and insect death occurs. The proteinases acting as immune inhibitors of the type A (InA) (Edlund T. et al., Infect. Immun. 14, 934-41; 1976), by degrading proteolytically the cecropin-like antibacterial proteins (Dalhammar G. & Steiner H., Eur. J. Biochem. 139, 247-52; 1984), could explain partly the mode of pathogenesis of insect bacterial infections. During parasitism in the honeybee, *B. larvae* excretes an extracellular proteinase of a character of immune inhibitor that suppresses selectively the inducible immune peptides in the bee haemolymph. Apidaecins and abaecin exposed to the action of *B. larvae* proteinase lost entirely antibacterial activity directed against *Escherichia coli*. Similar to other immune inhibitors released by insect bacterial parasites, the proteinase of *B. larvae* destroys specifically an antibacterial activity of cecropins from *Galleria mellonella*, *Celerio euphorbiae*, and activity of synthetic cecropin B (Serva) of *Hyalophora cecropia*. The AFB scales collected from various apiaries differ significantly in their proteolytic activity that coincides with the ability to inhibit antibacterial response proteins in insect immune haemolymphs. Preliminary identification indicates the similarities of the *B. larvae* exoproteinase to an immune inhibitor of the type A (InA) from *Bacillus thuringiensis* (Sidén I. et al., J. Gen. Microbiol. 114, 45-52; 1979). Evidence presented in this note seems promising in understanding way by which *B. larvae* could disturb non-self response system in the honeybee. By inhibiting the antibacterial response peptides, the multiplication of *B. larvae* in bee brood coelomic cavity is made easier.

14-091

A COMPARATIVE STUDY OF HYGIENIC BEHAVIOR IN SEVERAL HONEY BEE RACES

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Hygienic behavior was studied in four types of honey bees: Italians (*Apis mellifera ligustica*), Carniolans (*Apis mellifera carnica*), Africanized bees (wild type bees in Brazil, predominantly *Apis mellifera scutellata*), and hybrid Italian/Africanized bees (virgin Italian queens open mated with Africanized drones). The tests were done in Ribeirão Preto, in the state of São Paulo, Brazil. Each type of bee was represented by five four-frame nucleus colonies, with standard deep Langstroth frames, except for the "hybrid" bees, which included only three nuclei. Each group of colonies was tested five times, at intervals of 10 days. Hygienic behavior was induced by perforating the cell cappings of sealed worker brood with a needle, to a depth that would also pierce the enclosed pupa. The Africanized bees were the most efficient (P < 0.05, Tukey test). The Carniolan bees were the least hygienic among the four types of bees.

14-093

THE INFLUENCE OF DIET ON VITELLOGENIN AND TOTAL PROTEIN TITRES IN THE HEMOLYMPH OF CONFINED ADULT HONEY BEES (*APIS MELLIFERA*).

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A laboratory-based test of diet supplements for honey bees was made. It consisted of evaluating total protein and vitellogenin (Vg) in the hemolymph of bees confined in small cages in groups of 120, kept since emergence, at 34°C and 80% RH, and fed with 1) 1:1 beebread (pollen stored in the comb) and candy (honey mixed with sucrose), 2) 1:1 frozen pollen, previously collected from honey bee colonies and candy, 3) 40% soy meal, 10% sugar cane yeast (from an alcohol distillation plant), and 50% sucrose, 4) 1:1 fine corn meal and sugar, or 5) 50% sucrose syrup. Water was added to the powdered ingredients of diets 3 and 4 to form a thick paste. Additionally water was supplied *ad libitum*. At 0, 2, 4 and 6 days, total protein in the hemolymph was determined in 10 bees from each group, using a protein-binding dye assay. Vg was estimated, using rocket immunoelectrophoresis, in three pools of hemolymph, each prepared from four 6-day old bees. Two-day-old workers fed on beebread had a statistically greater amount of total protein than did those fed on frozen pollen, corn meal or sugar syrup. By the fourth day, workers maintained on frozen pollen diet demonstrated a recuperation of total protein titre, comparable to those fed on beebread. On day six, only the bees fed on frozen pollen had non-specific protein levels comparable to that seen in bees fed on beebread. The soybean - yeast diet was significantly better than corn meal or sugar alone, both for total protein and Vg. However this artificial diet, along with frozen pollen was inferior in terms of Vg, to the beebread. There was no Vg production with corn meal or syrup alone. Though soybean meal and yeast have a protein level similar to pollen, this diet does not promote normal protein secretion levels in honey bees. The laboratory tests proved to be useful for discriminating the utility of the diets tested. Research supported by CAPES.

14-094

INHIBITION OF FAT BODY PROTEIN SYNTHESIS BY JUVENILE HORMONE ANALOGUE (PYRIPROXYFEN) IN AFRICANIZED BEES

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Several studies have shown that juvenile hormones (JH) or their analogs can promote fat body protein synthesis in insects. Little is known, however, about the control of protein synthesis and secretion, particularly vitellogenin (Vg), during the adult life of honey bees. The aim of this study was to assess the action of a JH analog, Pyriproxyfen (PPN), on fat body protein synthesis in *Apis mellifera* workers.

Five groups of 120 newly emerged workers were topically applied with 0 (controls), 0.01, 1, 5 or 10 µg PPN in 1 µl acetone. These bees were kept confined at 34°C, 80% R.H. for 6 days and were fed on a diet (40% beebread, 10% honey and 50% sucrose) that permits normal synthesis of protein and Vg. Water was supplied *ad libitum*. Fat body and hemolymph from 20 workers, aged 0 to 6 days, randomly collected from each confined group, were pooled for protein-binding assay, SDS-PAGE and rocket immunoelectrophoresis. The quantitative data (four repetitions) were analyzed by Kruskal-Wallis test. Ultrastructure of fat body cells from treated workers and controls were compared by transmission electron microscopy.

Normal Vg accumulation in hemolymph during the first week of adult life does not occur in workers treated with 10 µg PPN. Low Vg ($p=0.02$) and protein ($p\leq 0.05$) titres were observed in 5 and 6-day-old treated workers. This result was confirmed by the faint Vg band in hemolymph on SDS-PAGE. Fat body of treated groups showed no alteration in the protein pattern, but a decrease in number and volume of protein granules was observed in 10 µg PPN-treated workers, demonstrating the inhibitory effect of the higher PPN dose on protein synthesis. The lower doses did not change fat body ultrastructure and did not cause any detectable effect on protein and particularly on Vg titres. In conclusion, the JH action in bees is related to inhibition and not activation of protein synthesis and secretion by fat body cells.

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14-096

CUTICULAR MELANIZATION AND PROPHENOLOXYDASE ACTIVITY INDUCED BY A JUVENILE HORMONE ANALOG IN *APIS MELLIFERA*

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In *Apis mellifera* a precocious and altered pigmented cuticle results from juvenile hormone analog (Pyriproxyfen - PPN) treatment. This hormonal control of melanization, described in some insects, was studied in worker pupae treated with 1 µg PPN, by measuring prophenoxydase (PO) activity.

Activity of the PO system, a key enzyme involved in pigmentation, was determined by homogenizing 15, 16 or 17 day-old pupae previously treated with PPN on day 11 or 12, in 400 µl of 0.01M sodium cacodylate buffer, pH 7.0 and 5mM calcium chloride. After centrifuging at 0-4°C and 10,000 x g for 15 min, the supernatant was poured through glass wool to remove floating fat particles, and used for the PO-activating assay. Filtered supernatant (50 µl) was incubated with 200 µl L-dihydroxyphenylalanine (saturated solution in water) and 450 µl cacodylate buffer. A few crystals of phenylthiourea (PTU) were added to some reaction mixtures. After 15 min at room temperature the absorbance at 490nm was measured in a spectrophotometer. All quantifications were made within the range in which reaction rates were proportional to enzyme concentration.

PO activity was higher in treated than in untreated pupae. Activity was strongly inhibited by PTU. The alteration of timing of cuticular melanization onset by the JH analog used, and the increased PO activity detected in PPN-treated bees demonstrated an effect of JH on the metabolic pathway leading to melanin synthesis in *Apis mellifera*.

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14-095

HEAT-SHOCK INDUCED PROTEINS IN FAT BODY CELLS OF *APIS MELLIFERA* CULTURED *IN VITRO*.

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Effects of heat shock in insects have been studied and special attention was given to protein synthesis after expositions to high temperatures. To obtain evidence concerning to temperature-induced proteins in worker bee, fat bodies from 5th instar larvae (feeding and spinning phases) were cultured *in vitro*. After rinsing in insect Ringer solution and incubation for 15-40 min in complete medium, the fat bodies were placed in 1 ml of incorporation medium containing 12.5 µCi [³H] leucine (167 Ci/mmol) for 2 h, with rotary shaking, at 34.5°C (controls) or 42°C. The medium was assayed for secreted radioactive proteins. Fat bodies were homogenized in 10% sorbitol for qualitative and quantitative studies of radioactive unsecreted proteins. Secreted and unsecreted proteins were concentrated by acid precipitation and counted by liquid scintillation. SDS-PAGE and fluorography were performed on precipitated samples of fat bodies and culture media. There was synthesis of temperature-induced proteins in fat bodies cultured at 42°C. Three bands corresponding to 29, 70 and 80 kDa polypeptides were detected in the fat bodies submitted to heat-shock. In fat body controls, maintained at 34.5°C these polypeptides were not detected. The temperature-induced proteins were only detected in the fat body cells, they were not secreted into the culture medium, and possibly were synthesized to protect cells against the harmful effects caused by high temperature. A possible analogy between temperature-induced *A. mellifera* 70 kDa protein and *Drosophila* 70 hsp is under investigation.

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14-097

IMPACTS ON HONEY BEES OF PROTEASE INHIBITORS *IN VITRO* AND IN TRANSGENIC OILSEED RAPE PLANTS.

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Protease inhibitor (PI) genes incorporated into transgenic plants may effectively protect against pest attack. However, their safety to honey bees must be established before field release. Serine protease activities predominate in the guts of larval and adult bees. *In vitro* tests in which caged adult bees were fed *ad lib* with 3 serine PIs in sugar syrup, BPTI (bovine pancreatic trypsin inhibitor), SBTI (Kunitz soybean trypsin inhibitor) and SBBI (Bowman-Birk soybean trypsin inhibitor), and a cysteine PI from rice, OCI (oryzacystatin I), showed that high concentrations (1-10 mg/ml) of BPTI and SBTI reduced the activities of gut serine proteases and also significantly reduced bee longevity. Longevity was not reduced among bees fed lower concentrations (10-1000 µg/ml) of BPTI and SBTI, or those fed SBBI and OCI at 26 µg/ml. SBBI and OCI did not alter serine protease activity levels in bees fed PI at 26 µg/ml for 15 days. Bees fed 11 µg of SBBI or cystatin (cysteine PI from egg white) over 24h consumed food at a normal rate and showed less than 10% mortality 1-3 days after dosing. Addition of SBBI or OCI (1, 5, 10 µg/ml) to the reward offered to bees in a conditioned proboscis extension assay showed that the PIs did not affect short-term learning ability in 15-day-old bees. Continuous feeding with these PIs (26 µg/ml) for 15 days did not affect their olfactory learning ability. Nectar analysis of 2 lines of OCI-transgenic oilseed rape plants showed that plants from the higher expressing line secreted a greater volume of nectar with a higher sugar content than the controls. However, bees did not show any preference for these plants when foraging behaviour patterns were observed.

14-098

NON-ADDITIVE EFFECTS ON HONEY BEE (*A. MELLIFERA*) COLONY PERFORMANCE BY ARTIFICIAL INSEMINATION OF QUEENS WITH MIXED SEMEN FROM DRONES OF SEVERAL BEE LINES (APIDAE: HYMENOPTERA)

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The performance of the bee colony as a whole is determined by the cooperation of thousands of individual workers. Due to the multiple mating of the honeybee queen, workers may differ in characteristics relating to the genetic properties of their respective fathers. The result of a cooperation between these workers might be specific to the particular combination and might deviate from what would be expected from a mere summation of the performance of the genetic types between themselves.

It was investigated whether synergistic effects would occur in colonies headed by queens which were inseminated with drones from several breeder lines. Sperm was mixed using either drones from the same line or using equal numbers of drones from all of the 3 or 4 breeder lines. In most of the the measured colony parameters there was no clear difference between the performance of the combined group and the mean calculated from the single groups. There was, however, a consistend trend towards lower performance in traits relating to disease control, as hygienic behavior or defending against the parasite *V. jacobsoni*.

14-100

THE INSECT GROWTH REGULATOR FENOXYCARB AFFECTS LARVAL GROWTH RATE, LARVAL DEVELOPMENT AND K⁺-DEPENDENT MIDGUT AMINO ACID ABSORPTION IN THE SILKWORM *BOMBYX MORI*

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Fenoxycarb is a potent non-neurotoxic insect growth regulator (IGR) which induces a non-spinning syndrome in the silkworm *Bombyx mori*. Larvae are particularly sensitive when exposed during the last instar. Fifth instar larvae were force-fed or topically treated with different amounts of the IGR (from 10⁻¹⁵ to 10⁻⁶ g/larva). Prolongation of feeding period, reduction of growth rate, dose-dependent increase in number of dauer larvae was observed with doses higher than 10⁻¹² in both treatments. Instead, a dose-dependent frass reduction was seen only in topically treated insects, while a significative and constant inhibitory effect was obtained from an ingested dose of 10⁻¹⁵.

Midguts, divided in their anterior-middle (AM) and posterior (P) regions, were removed from treated larvae at day 6 and brush border membrane vesicles (BBMV) prepared. Labelled leucine uptake with time was measured in the presence of K⁺-gradient, that provides the driving force for intravesicular amino acid accumulation. Despite some differences with the two treatments, the main results indicate an increase of leucine uptake and accumulation, especially in AM-BBMV, with an IGR dose of 10⁻¹⁵, suggesting a hormone-like effect. Conversely, a dose of 10⁻⁶ causes a reduction of both parameters, possibly an aspecific toxic effect of the carbamate, involving the transport protein or the characteristics of the membrane lipid bilayer.

14-099

REPRODUCTIVE SUCCESS OF *APIS MELLIFERA SCUTELLATA* IN THE AMERICAS

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Africanized honey bees, the progeny of *Apis mellifera scutellata* in the Americas, has spread from central Argentina in South America to the southern United States in North America in less than 40 years. This remarkable range expansion has been the subject of numerous research efforts to determine the underlying ecological, behavioural and genetic processes. The antecedents of the range expansion can be found in the natural history of *Apis mellifera scutellata*. The African honey bee traits of high swarming rates, nomadic behavior, intensive absconding, and a "k-selected" demography all combined with the highly favorable tropical environment of South America to produce the remarkable population explosion of Africanized honey bees. Differences in ecologically based population cycle regulators allowed Africanized honey bees to expand populations where European honey bees could not survive. The genetic processes have been less easy to discover but persistent efforts now indicate that hybridization, differential fitness, and selection have all played a role.

14-101

METABOLIC FATE OF ECDYSONE INJECTED INTO DIAPAUSE EGGS AND NON-DIAPAUSE EGGS OF THE SILKWORM, *BOMBYX MORI*

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It has been demonstrated that silkworm eggs contain various ecdysteroids and that the amounts of ecdysteroids differ between diapause eggs and non-diapause eggs. In the present study, in order to compare the metabolism of ecdysone between diapause eggs and non-diapause eggs, we injected [3H]ecdysone into two types of eggs at various stages of embryonic development. The resultant radioactive metabolites were analyzed by reverse-phase HPLC.

A significant difference in ecdysone metabolism between diapause eggs and non-diapause eggs was detected from the late stage of gastrulation (48-hr embryo), at which stage the embryos enter into diapause. In the diapause eggs, most of the radioactivity was detected in the conjugated ecdysteroid fraction (mainly 3-epiecdysone 22-phosphate). By contrast, in non-diapause eggs, scarcely any radioactivity was detected in the conjugated ecdysteroid fraction, and most of the [3H]ecdysone was efficiently converted to 3-epi-20,26-dihydroxyecdysone. These results suggest that phosphorylation of ecdysteroids is major pathway in diapause eggs, while, hydroxylation at C-20 and C-26 of ecdysteroids is the dominant metabolic pathway in non-diapause eggs. Although the causal relationship between the initiation of embryonic diapause and phosphorylation of ecdysteroids is obscure at present, our present results suggest the possibility that a marked increase in phosphorylation of ecdysteroids causes the embryos to enter into the embryonic diapause.

14-102

THE CHANGE OF SORBITOL CONTENT IN *BOMBYX MORI* EGGS DURING CONTINUOUSLY INCUBATION AT 25°C (LEPIDOPTERA: BOMBICIDAE)

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Diapause of silkworm, *Bombyx mori*, eggs is initiated by exposure at 25°C for several days after oviposition, but the longer period of 25°C incubation cause the fewer number of hatchable eggs. To understand why long term incubation at 25°C causes a decline of the hatchability of *B. mori*, we investigated a change of the content of sorbitol, as an index of embryonic diapause, in some strains of the eggs during continuously incubation at 25°C.

As a result, the sorbitol content was decreased in all strains of the eggs during 25°C incubation. However, the curves of the degradation were categorized into 2 types.

We will discuss a relationship between the degradation of sorbitol content in *B. mori* eggs during continuously 25°C incubation and their hatchability.

14-103

EVALUATION OF ENZYMATIC ACTIVITY IN HONEY PRODUCED BY *APIS MELLIFERA* L.

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The honey enzymes diastase and invertase are generally considered as being of animal origin. However a number of researches on the characterization of Italian unifloral honeys showed that the enzyme content is stricly depending on the honey botanical origin.

In this study diastase and invertase content were determined on a large sampling of unifloral honeys from the following botanical sources: *Arbutus*, *Carduus*, *Castanea*, *Citrus*, *Erica*, *Eucalyptus*, *Hedysarum*, *Helianthus*, *Rhododendron*, *Robinia*, *Rosmarinus*, *Taraxacum*, *Thymus*, *Tilia*, fir honeydew and honeydew produced by *Metcalfa pruinosa*.

The honey types studied showed a considerable difference in their enzyme content. In particular, *Arbutus*, *Robinia*, *Erica*, *Citrus* and *Rosmarinus* honeys are characterized by the lowest values; *Thymus*, *Eucalyptus*, *Castanea* and the two honeydew honeys by the highest values.

The observed variability may be probably due to a series of different intrinsic and extrinsic factors as, for example, the period of nectar flow in relation to the biologic cycle of the honey bee family.

This high variability among the different honey types must be taken into account when these parameters are used to establish honey freshness.

Section 15
Agricultural Entomology

15-002

PUTTING THE PIECES TOGETHER: FINE-SCALE MOVEMENTS AND LARGE-SCALE DISTRIBUTION OF A FOREST DAMSELFLY IN AGRICULTURAL LANDSCAPES.

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The forest damselfly *Calopteryx maculata* has two focal resource patches - streams for mating and oviposition and forested areas for foraging. Animals fly between these patches, linking the resources within the landscape. In pre-dominately agricultural landscapes, animals must move farther to access the same set of resources. I experimentally manipulated damselflies in forested and agricultural landscapes to measure connectivity as a function of landscape type. Simulation models were then used to predict medium- and large-scale patterns of distribution from fine-scale movements and predictions were tested against empirical data from Ontario and Nova Scotia. Landscape structure alters patterns of movement at fine- and medium- spatial scales, however, these patterns are not necessarily manifested in altered distributions at larger spatial scales.

15-001

THE EFFECTS OF FEEDING PROTEASE AND α -AMYLASE INHIBITORS, SINGLY AND IN COMBINATIONS TO THREE SPECIES OF LEAFROLLERS (LEPIDOPTERA: TORTRICIDAE)

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The larvae of three leafroller species - *Epiphyas postvittana* (Walker), *Planotortrix octo* (Dugdale) and *Ctenopseustis obliquana* (Walker) - were chronically fed six digestive enzyme inhibitors in artificial diets over a two-week period from hatching, and their growth rate and digestive enzyme activities were measured. The inhibitors were fed singly and in combinations set to target most of the digestive enzyme groups present in these leafrollers. Of the four protease inhibitors (PIs), three were extracted from potatoes (POT1, 2, and 4) and the fourth was bovine pancreatic trypsin inhibitor (BPTI). The two α -amylase inhibitors (α AI) were extracted from wheat, the dimer (WD) and tetramer (WT). Previous trials had shown these inhibitors to be the most effective from a range of 21PIs and 6 α AI.

POT1 significantly affected the growth rate of all three leafrollers and BPTI was effective against *E. postvittana* and *P. octo*. Most combinations of inhibitors did not reduce the growth rate further than the single most effective constituent, i.e. inhibitors presented singly in the diet were generally more effective in reducing the growth rate. One exception to this was a combination of POT1, BPTI and WT against *P. octo*.

All digestive enzyme activity was affected by the presence of the inhibitors in the diets. α AI increased α -amylase activity between 1.5 and 10 times. BPTI, either singly or in combination, dramatically reduced trypsin activity (10 times) and raised elastase activity (about 1.5 to 3 times) in all species. Since larval growth rate was so little affected whilst all enzyme activities were greatly altered, the question of compensation of one digestive enzyme for another, is discussed.

15-003

EFFECTS OF HABITAT FRAGMENTATION ON PLANT-INSECT COMMUNITIES

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The fragmentation of habitats is a major threat to near-natural ecosystems in the agricultural landscape, resulting in both decreasing area and increasing isolation of the remaining fragments.

Investigations were conducted using the leguminous plant species *Trifolium pratense* and *Vicia sepium* and the associated insect-communities consisting of endophagous herbivores and their parasitoids.

Effects of fragmentation were analyzed on near-natural meadows of different size and isolation near Karlsruhe, southwestern Germany. Insect diversity on both *T. pratense* and *V. sepium* was positively correlated with habitat size. On small meadows loss in both diversity of insect species and number of specimens was higher for parasitoids than for endophagous herbivores. Local extinction was more severe for rare species than for abundant ones. Furthermore, species with small populations showed a higher variability in their abundances between populations than species with large populations.

The effect of isolation on colonization-success of insects was analyzed experimentally on manually created plant islands. The experiments showed a decrease in insect diversity with increasing isolation of the plant islands. Abundant species colonized more islands than rare ones. Colonization-failure was higher for parasitoids than for herbivores. Thus, isolation of the plant-islands led to a significant reduction in parasitism of the endophagous herbivores and to imbalance of herbivore-parasitoid interactions.

15-004

THE IMPACT OF UNCULTIVATED CORRIDORS ON ARTHROPOD POPULATIONS WITHIN SIMULATED AGROLANDSCAPES

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We learned during the 1980s that biotic diversity cannot be conserved by a single-species approach; we hopefully will learn during the 1990s that we cannot sustain agricultural productivity by a single-field (agroecosystem) approach. Rather, as agrolandscape approach must be adopted in which landscape elements (e.g., patches and corridors) are patterned to optimize for a set of parameters such as insect pest control, nutrient retention, habitat fragmentation, net energy, trophic diversity, and crop productivity. An experimental approach is necessary to evaluate these factors in an replicated (simulated landscape) research design.

Results from several investigations indicate that uncultivated grassy corridors within soybean agroecosystems reduced the densities and affected distribution of adult potato leafhoppers (*Empoasca fabae*), and increased the rate of infestation of the green cloverworm (*Plathypena scabra*) by the frugal pathogen (*Nomvraea aileyi*). Uncultivated successional corridors, however, failed to "funnel" predators into the soybean crop.

Cultivated corridors planted in either tall or short sorghum (*Sorghum bicolor*) significantly reduced densities and the rate of dispersal of the Japanese beetle (*Popillia japonica*) in intercropped soybean agroecosystems. Patch architecture and corridor "associational resistance" functioned as mechanisms to regulate populations of insects pests. Findings from these studies suggest that integrated pest management should be investigated and implemented at the agrolandscape temporal-spatial scale.

15-006

THE ROLE OF NATURAL WATER CATCHMENTS ON THE PRODUCTION OF VECTORS OF PLANT VIRUSES IN THE ARAVA VALLEY, ISRAEL

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The timing and quantity of insect pests invading intensive agriculture is important to understand, and predicting these events is critical to developing successful control strategies. Leafhoppers, aphids, thrips and whiteflies, taxa known to contain plant virus vectors, were studied over a two-month period (March-April 1995) in natural, xeric habitats near intensive agricultural settings of the Arava Valley, Israel. The study was carried out to determine if the timing and quantity of emigrants were differentially influenced by the moisture regimes of three habitats, separated by less than 1 km. These habitats were monitored for target taxa using a variety of collecting devices: yellow pans; malaise traps; small, battery-powered suction traps; and emergence traps. Yellow pans proved useful for capturing aphids and thrips; malaise traps collected large quantities of leafhoppers and aphids; the small suction traps, operated only occasionally, were excellent for monitoring thrips abundance just above ground level; and emergence traps captured target taxa erratically and proved to be of limited value during this study. Whiteflies were scarce during the two-month study, but the other 3 taxa were abundant. Several thousand specimens within the target insect families were collected and are being curated and identified to species. The composition of taxa captured in the three habitats was different. The possible reasons for these difference is discussed.

15-005

INTERCHANGES OF A COMMON PEST GUILD BETWEEN ORCHARDS AND THE SURROUNDING ECOSYSTEMS: A MULTIVARIATE ANALYSIS OF LANDSCAPE INFLUENCE

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Landscape quality and structure should be an important factor to explain insects distribution and activity, at a regional or local scale. The measurement of faunal exchanges occurring between an agroecosystem and its surrounding habitats is relevant to test landscape influence. But in cultivated landscape, management practices may be an important factor influencing the exchanges between ecosystems. The activity of tortricids (Lep. Tortricidae), flying between apple orchards and their surrounding habitats were used to test this hypothesis in the region of Nyon - La Côte (Switzerland). A Malaise trap was placed on each of the North, East, South and West margins of six apple orchards of two types (three IPM and three abandonned). Two collectors were placed on top of the traps to separate emigrant from immigrant individuals. From the beginning of June to October 1992 and 1993, 48 samples were collected on 14 successive weeks.

There were a high similarity (Mantel test for multivariate data) between emigrant and immigrant tortricid captures, if they were caught by the same Malaise trap. We conclude that there were neither emigration nor immigration but an activity of tortricids at the interface of habitats.

The hypothesis of the landscape influence on tortricids activity at the interface was tested at two scales. The first concerned the proximate surroundings of the orchard and the second the distant surroundings (300m.). Proximate and distant surroundings were assigned to 6 categories including woodland, vineyard, cereal crop, orchard, garden and meadow. At both scales, multivariate analysis (Correspondence Analysis, Canonical Correspondence Analysis) and associated statistics (multiple regression and canonical coefficients, Monte Carlo permutations test), showed that landscape explained a significant part of the variation of tortricids caught at the interface between both types of apple orchards.

15-007

EFFECT OF HABITAT HETEROGENITY ON DIVERSITY AND DENSITY OF POLLINATING INSECTS (HYMENOPTERA, APOIDEA)

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Diversity and density of wild bees *Apoidea* were studied in different landscape ecosystems, i.e. in bee refuges (forests, shelterbelts, natural swards and field margins) and on selected plantations (red clover, alfalfa, sunflower, winter rape, yellow lupine, buckwheat, flax). Special analysis was carried out for several forest islands of various sizes which indicated that they are inhabited by bee communities consisting of many species. Densities of these insects were higher than anywhere else.

Diversity and density of wild bees in two types of agricultural landscape with complex and simplified structure in Poland and Romania were compared. It was found that in the complex landscape the density of wild bees is 3-4 times higher. Also the diversity of wild bees in this landscape is higher.

The rules of occurrence in landscape were studied. It was indicated that one of the factors compensating for reducing effect of economy on wild bees populations is mosaic structure of landscape.

15-008

AGRICULTURAL TRANSFORMATION AND ANT DIVERSITY: THE CASE OF COFFEE IN THE NEOTROPICS

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Traditionally, coffee has been produced in a diverse agroforestry system that includes a diverse canopy of shade trees in addition to fruit trees and other crops. Over the last 50 years many coffee plantations in the neotropics have been converted to plantations with a low density and diversity of shade trees, or to coffee monocultures. Ants (Hymenoptera: Formicidae) were used as an indicator group to examine the effect of changes in vegetational complexity associated with the transformation of the coffee agroecosystem in Costa Rica. Both within and between-habitat diversity were examined to gain insights about the consequence of the transformation at the local and landscape levels. Ant diversity is significantly reduced as the agroecosystem is simplified, and patterns of species similarity suggest that the loss of species diversity at the landscape level could be even more dramatic. Mechanisms responsible for the diversity decline include direct ones, such as changes in microclimatic conditions and loss of nesting sites, and indirect ones, such as changes in species interactions among the ground-foraging ant community.

15-010

MOVEMENT BETWEEN AGROECOSYSTEMS AND SURROUNDING HABITATS: A DETERMINANT FACTOR IN THE POTENTIAL MANIPULATION OF NATURALLY OCCURRING PARASITIDS

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Naturally occurring parasitoids are frequently unable to maintain pest species at densities below the economic threshold. One possible explanation for this poor performance is the asynchrony of agroecosystem colonization by herbivores and their primary parasitoids and we will discuss the results of two different studies addressing this subject: one in tobacco the other in potato. In both cases there is clear evidence that spring emergence of the parasitoids occurs significantly earlier than their appearance in the agroecosystems. Furthermore, in the tobacco study there is appreciable asynchrony between emergence dates of the parasitoid and the pest herbivore. Therefore, a few generations must be completed in habitats surrounding the agroecosystems by exploiting an alternate host and/or the same host but on other plant species. The feasibility of various management practices proposed to increase the efficacy of these naturally occurring control agents will be discussed in the context of between habitat movement at the beginning and the end of the season.

15-009

PREDATOR MOVEMENT PATTERNS AND THEIR RELATION TO HABITAT STRUCTURE

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We have found large variation in the size and fecundity of individual *Pterostichus cupreus* L. from different field populations located within a small geographical area. Some populations are larger and more fecund than others. By using comparisons with laboratory experiments on food limitation we conclude that field variation is partially due to different resource levels.

We are interested in testing hypotheses as to the causes of this variation. Differences in the surroundings, for example field edges and field size, of the different populations and how they may affect movement are considered. We are using individual based movement models in order to estimate correspondence between the distribution of resources and the dispersal of the beetles. Comparisons of diffusion rates into and out of areas with different levels of resources are considered.

15-011

PARASITOID COMMUNITY STRUCTURE: IMPLICATIONS FOR BIOLOGICAL CONTROL IN AGRICULTURAL LANDSCAPES

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The community of parasitic Hymenoptera attacking each of 45 Lepidopterous pests of corn, soybean, wheat and maize in the North Central Region of the US was determined. The impact of herbivore host range, host plant type, feeding niche and host plant chemical defense on the species richness and relative proportion of generalist versus specialist parasitoids was examined.

Results indicate that exposed-feeding, polyphagous herbivores predominate in these crops and that generalist parasitoids make up the largest proportion of the parasitoid community (42%). Parasitoid richness is greatest on polyphages with generalist parasitoids equally common (ca. 50%) regardless of herbivore feeding breadth. Exposed feeders have the most parasitoids, while concealed feeders have relatively more specialists. Herbivores of monocots have the fewest parasitoids with a higher proportion of specialists and host plant chemical defense did not significantly affect parasitoid species richness. Because of the predominance of generalist parasitoids, the host habitat of their alternate hosts was determined. Over 60% of these species utilize alternate hosts that feed on mid or late successional plants. This indicates the potential importance of maintaining these non-crop habitats in the conservation of parasitoid communities in agricultural landscapes.

15-012

THE USE OF INVERTEBRATES IN EVALUATING RURAL SUSTAINABILITY

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Evaluate rural landscape sustainability is a main topic of western countries. The use of species abundance, diversity and distribution appears an useful tool to evaluate and compare different farming systems and different rural landscapes. Basic resources of diversity (invertebrate diversity) are allocated and supported by field margins, including hedgerows and uncultivated borders. Evaluate these reservoirs and develop an appropriate research method is crucial.

Some sampling methods, including broad or narrow taxonomical categories are discussed. Some key groups will be discussed as well. Comparison and evaluation of different peach orchards and apple orchards under decreasing pesticide input are analysed. Biological orchards compared with integrated and conventional ones support higher number of invertebrates species.

15-014

WHEN THE INFLUENCE OF LANDSCAPE PATTERNING SHOULD MAKE THE GREATEST DIFFERENCE

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While it is widely recognized that patchiness or heterogeneity of habitat can dramatically influence insect population dynamics, few field experiments or theoretical explorations have yielded practical guidelines for use in applications. I performed a field experiment in which plots of broccoli (*Brassica oleraceae*) were mixed with natural vegetation (an assortment of weeds) in long strips which varied in (1) proportion consisting of crop, and (2) absolute size of crop/weed patches. This design allowed me to examine the effects that both the pattern and the scale of landscape heterogeneity have on insect populations in an agroecosystem. Response variables were densities of herbivores -- cabbage aphids (*Brevicoryne brassicae*), flea beetles (*Phyllotreta cruciferae*) and cabbage butterfly larvae (*Pieris rapae*) -- sampled from broccoli plants in each of the different strips/treatments. While cabbage aphids showed a response strictly to proportion of strip consisting of crop, flea beetles were impervious to differences in crop proportion, but were strongly affected by scale, exhibiting further a sensitivity to an interaction between the two main effects. These results demonstrate that heterogeneity means different things to different insects, something which bears further examination. A model linking dispersal of the different insects to the spatially distinct treatments will be discussed as a way of predicting when pattern or scale of heterogeneity might be effective in influencing pest densities. This leads the way to developing a protocol for future predictive efforts based on measuring important behavioral insect characteristics such as dispersal.

15-013

THE IMPACT OF FIELD BOUNDARY HABITATS ON THE DIVERSITY AND ABUNDANCE OF NATURAL ENEMIES IN CEREALS

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The winter assemblage of predatory Coleoptera and Araneae was investigated in soil and vegetation samples in field boundaries around cereal fields. The assemblage was compared with the spring distribution of predatory arthropods in the field-margin zone and further into cereal fields.

The diversity and abundance of predatory species in cereal fields was greater within 10 m of the field boundary in spring and summer but not all the species were detected overwintering in the field boundary during the previous winter. The reasons for the apparent discrepancies in species composition are discussed in relation to the pattern of arthropod distribution, measurements of crop development and microclimate gradients out from field boundaries. The profile of predatory pressure of the arthropods was measured out to 50 m from field boundaries.

These results are used to interpret the relative merits of field management promoting a wider diversity of predators versus measures resulting in greater abundance of fewer species of more effective predators of aphids.

15-015

MOLECULAR BASIS OF CUCUMOVIRUS TRANSMISSION BY APHIDS

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Cucumber mosaic virus (CMV) and other members of the cucumoviruses are transmitted by aphids in a nonpersistent manner. Viral genomic RNAs 1, 2 and 3 are encapsidated in separate icosahedral particles. A successful transmission event requires the delivery of at least three virus particles to the same cell. Genetic analyses have shown that the primary determinant of aphid transmission maps to CMV RNA 3, and specifically to the encoded coat protein gene. Further analyses were made using infectious viral RNAs transcribed from a set of cDNA clones. Mutations were introduced in the coat protein gene in order to identify specific amino acids which play a role in aphid transmission. The transmission of a transmission-defective mutant by the aphid *Aphis gossypii* was restored by two nucleotide substitutions affecting encoded amino acid changes in two regions of the coat protein gene. Surprisingly, these same changes did not restore efficient transmission by a second aphid, *Myzus persicae*. Additional changes in the coat protein gene were required to restore efficient transmission by this aphid. Structural and immunological studies are underway to establish if the critical amino acids are present on the surface of virions, or if they influence transmission indirectly by altering secondary structure or physical properties of virions. Monoclonal antibodies are being used to differentiate mutants with single amino acid changes in the coat protein.

15-016

MOLECULAR BASIS OF POTYVIRUS TRANSMISSION BY APHIDS

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Transmissibility of potyviruses by their aphid vectors is dependent upon the presence of appropriate amino acids in a domain near the N-terminus of the capsid protein (CP) as well as a functional helper component (HC) protein. Mutational analyses with tobacco vein mottling virus (TVMV) confirmed the importance of an asp-ala-gly (DAG) sequence near the CP N-terminus, but further studies with TVMV and tobacco etch virus (TEV) have shown that alteration of the context within which the DAG lies also affects virion transmissibility.

The HC protein is thought to function by binding to aphid mouthparts and to virions, allowing the retention required for subsequent virion egestion and inoculation. The effect of mutations, in either the CP or HC, on the fate of virions in *Myzus persicae* was studied by immunogold and radioisotope labeling. Mutations, in either the CP or HC, which abolished aphid transmissibility also resulted in lack of virion retention in the food canal of the stylets. Our interpretation of these results is that the defective HC mutants either fail to bind to the aphid, fail to bind to virions, or bind to neither. The transmission-defective CP mutants fail to bind to HC. Either process leads to lack of virion retention in the food canal and hence lack of transmission. The results of *in vitro* and *in vivo* experiments to obtain direct evidence of a double binding role for HC will be presented.

15-018

MORPHOLOGICAL AND CELLULAR BASES OF VIRUS TRANSMISSION BY WHITEFLIES

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Preparation of Whiteflies for Microscopy and Immunocytochemistry. A protocol was established for embedding whole insects in LR White at a sufficiently moderate temperature to preserve viral antigenicity. The four-step protocol includes infusing the resin with N₂ gas, evacuating or degassing the N₂-infused resin, covering embedding molds with Aclar®, and polymerizing the resin in a N₂ atmosphere at 55°C.

Whitefly Morphology and Noncirculative Virus Transmission. The combined afferent duct, cupula, piston, efferent duct and maxillary saliva canal of *Bemisia* can be likened to a hypodermic syringe in both form and function. Saliva exiting the pump, via the efferent duct, enters the salivary canal where food from the maxillary food canal enters the antecibarium. The roof of the latter is formed by the epicibarium (inner wall or sclerite of the clypeus) and its floor by the hypocibarium (inner wall or sclerite of the hypopharynx). The postcibarium (cibarial or "sucking" pump) of the feeding apparatus functions as a reversible bellows, allowing for egestion as well as ingestion. The latter morphology suggests that noncirculative whitefly-borne viruses are "cuticula-borne": acquired by ingestion, carried at specific sites on the cuticula lining of the feeding apparatus (maxillary food canal, precibarium, cibarial valve and postcibarium) and inoculated to plants via egestion.

Localization of Geminiviruses in Plants and Whiteflies. The aforementioned N₂-infusion embedding technique and elucidation of the internal morphology of *Bemisia* enabled us to localize SLCV geminivirus in plants by light microscopy and silver-enhanced immunogold staining and in whiteflies by electron microscopy and immunogold labeling. Geminiviruses are circulative and presumably inoculated to plants in watery saliva excreted into the phloem during "declogging" activity to clear the lumina of the feeding apparatus.

15-017

MOLECULAR BASIS OF CAULIMOVIRUS TRANSMISSION BY APHIDS

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Semi-persistent aphid transmission of cauliflower mosaic virus (CaMV) is dependent on the presence of a virus-encoded 18 kDa polypeptide (P18), designated aphid transmission factor. CaMV gene II, which encodes P18, was expressed in Sf9 insect cells using a recombinant baculovirus. Aphid transmission factor activity was recovered from these cells. Native P18 accumulates in Sf9 cells as characteristic paracrystals which are also found in CaMV infected plants. The active form of P18 can be solubilized from paracrystals and the equilibrium between the two states is influenced by calcium concentration. Therefore, it is suggested that paracrystals act as reservoirs for the solubilized active aphid transmission factor.

A current hypothesis is that aphid transmission factors act as molecules bridging between virions and vectors, *via* two distinct domains. The C-terminal P18 domain (31 amino acids) was consistently demonstrated to mediate a specific binding to virus particles. Since P18-virion binding alone is not sufficient to explain transmission, the putative involvement of additional P18 properties (such as microtubule-binding) in the molecular mechanisms of CaMV aphid-transmission will be discussed.

15-019

MOLECULAR BIOLOGY OF WHITEFLY-BORNE CLOSTEROVIRUSES

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A number of RNA plant viruses are transmitted by whiteflies. Of these, lettuce infectious yellows closterovirus (LIYV), transmitted by *Bemisia tabaci*, the sweet potato whitefly, is the best studied and well characterized. The LIYV genome is composed of two single-stranded RNAs of 8,118 (RNA 1) and 7,193 (RNA 2) nucleotides. Computer-assisted sequence analyses suggest that LIYV RNA 1 encodes for the principal replicative proteins, while RNA 2 contains 7 ORFs and includes the hallmark closterovirus gene array. Despite being bipartite, the LIYV genome size and overall organization are very similar to the monopartite genomes of the aphid-transmitted beet yellows closterovirus and citrus tristeza closterovirus, and some of the encoded proteins show significant levels of similarity. Full-length cDNA clones representing LIYV RNAs 1 and 2 have been constructed and transcripts have been generated *in vitro* and used to successfully infect tobacco mesophyll protoplasts. Interestingly, LIYV RNA 1 is capable of replication in the absence of RNA 2. Although LIYV replicates to high levels in protoplasts, we have so far been unsuccessful in transferring LIYV from protoplast extracts back to plants by using *B. tabaci*. A RT-PCR assay was developed based on predicted, conserved nucleotide sequences for LIYV and other closteroviruses and used to detect other whitefly-transmitted closteroviruses. LIYV appears to be representative of a group of emerging whitefly-borne closteroviruses.

15-020

MOLECULAR BASES OF LUTEOVIRUS TRANSMISSION BY APHIDS

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Beet western yellows luteovirus (BWYV) is transmitted in a circulative, non-propagative manner by aphids. Virions contain a minor protein, P74, produced by translation extension of the coat protein (CP) cistron into the neighboring readthrough domain (RTD). We have previously shown that the RTD contains determinants essential for BWYV acquisition/transmission by *Myzus persicae*. Subdomains within the RTD are being functionally characterized by site-directed mutagenesis followed by aphid transmission assays. Inoculum was delivered to aphids as purified virus, leaves of agro-infected plants or extracts of transcript-infected protoplasts. In-frame deletions in the C-terminal, variable half of the RTD generally did not abolish acquisition/transmission but greatly reduced its efficiency. By contrast, the N-terminal, conserved half of the RTD appears to be largely essential for acquisition/transmission. Deletion of the proline hinge between the CP and the RTD abolished incorporation of P74 into virions and no acquisition/transmission was observed. The effects of RTD mutations on accumulation of virus in agro-infected plants and tests for a potential role of other viral non-structural proteins in the acquisition/transmission process will also be discussed.

15-021

APHID AND VIRAL FACTORS REGULATING THE CIRCULATIVE TRANSMISSION OF SUBGROUP 1 LUTEOVIRUSES

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The barley yellow dwarf luteoviruses (BYDV) are obligately transmitted by aphids in a circulative, nonpropagative manner and efficient transmission is often limited to one or two specific aphid species. The transmission process requires that virions traverse the aphid hindgut epithelial cells into the hemocoel and then traverse accessory salivary gland (ASG) cells into the salivary canal. Virus exits the aphid along with salivary secretions during feeding. Ultrastructural studies suggest that the passage of BYDV virus particles across the plasmalemma of hindgut and ASG cells occurs by receptor mediated endocytosis. In addition, virus must bind to, and move across, the basement membrane surrounding the ASG; the mechanisms of which are unknown. The membrane that serves as a barrier to transmission in non-vector aphids differs for various virus isolate-aphid species combinations suggesting that different virus capsid protein domains interact with putative receptors on the three aphid membranes.

The BYDV capsid is composed of two proteins; a 22-kDa coat protein (CP) and a minor readthrough protein, (RTP). The RTP contains the CP sequence fused to a 50-kDa C-terminal extension, encoded by ORF5 and expressed by translational readthrough of the CP gene termination codon. Preparations of luteoviruses purified from plants contain a truncated form of the RTP which lacks the C-terminal portion of the 50-kDa domain. Various genetic modifications in the RTP coding sequence indicated that the RTP is not required for assembly of luteovirus particles or for plant infection. The C-terminal portion is not required for transmission, but may influence virus movement in plants. The N-terminal portion is not required for virus movement through the hindgut, but is required for virus to move through the ASG associated membranes.

15-022

MOLECULAR BASIS OF TOSPOVIRUS TRANSMISSION BY THRIPS

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Among plant viruses, tospoviruses take a special position with respect to morphology, taxonomic position and transmission. The success of tomato spotted wilt virus (TSWV), the type species of the tospoviruses, is based on its property to be persistently transmitted by thrips and by its broad host range (which seems to be defined by the feeding preference of the vector, rather than other (host plant) determinants). The current (re-)emergence of TSWV is caused by the worldwide spread of its most potent vector, the western flower thrips *Frankliniella occidentalis*. For proper and environmentally safe control, knowledge about the virus-vector interactions is needed. Therefore we started to study the molecular determinants, both of the virus and the vector, which are essentially involved in transmission. Immuno-localizing of the NSs protein of TSWV in midgut muscle and salivary cells shows that TSWV replicates in its vector. It has no pathological effect on the vector and is not transovarially transmitted. Acquisition studies using various developmental stages revealed that only L1 larvae are able to retain the virus. Ingestion of virus by L2 or later stages did not result in virus replication. This finding suggests the existence of a receptor in the midgut of L1 larvae solely. By serial passage of TSWV on plants, mutants were obtained which were defective in transmission but still able to infect plants at wildtype rate. These mutants do not anymore produce the two envelope glycoproteins (G1 and G2), suggesting their crucial involvement in acquisition by the thrips. Inspection of the sequences of tospoviral glycoproteins reveals the presence of a conserved RGD ("cell attachment") motif in the exposed N terminus of G2 and this motif may represent the receptor binding site of these viruses.

15-023

LOCAL AND GLOBAL EPIDEMIOLOGY: INTERACTIONS OF VECTORS, PLANTS AND CLIMATE WITH THE PATHOGEN XYLELLA FASTIDIOSA

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Xylem-sap-feeding hemipterans transmit the bacterial pathogen, *Xylella fastidiosa*, to numerous species of plants, causing disease in numerous perennials such as grape, lucerne (alfalfa), *Prunus* species, oak, elm, and citrus. These diseases are limited to the Americas but with recently reported detections in Europe and Taiwan. Can we predict future expansions of the range of *X. fastidiosa* or to new crops? Because of the very broad range of competent vectors, a lack of potential vectors should not restrict the potential geographic range of the bacterium. Winter climate appears to limit the geographic range of *X. fastidiosa*, based on its reported distribution, experimental cold therapy, and the geographic distributions of disease resistance of indigenous plant species. It is proposed that climate governs rates of plant recovery from acute infections. Regionally and locally, vector distribution and behavior and the persistence and systemic movement of *X. fastidiosa* in reservoir plant hosts are the critical determinants of infection rates and thus disease intensity. Implications for strategies for local control, prevention, exclusion and the rapid evolution of new diseases of diseases caused by *X. fastidiosa* will be discussed.

15-024

THE ROLE OF APHIDS AS VECTORS IN THE EPIDEMIOLOGY OF A STONE FRUIT VIRUS: PLUM POX VIRUS (PPV).

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PPV is a nonpersistent aphid-borne virus. It is the only virus transmitted by this way which causes serious economic problems in fruit tree crops. Since it was first seen in Bulgaria (1915), it progressively extends its geographical area. Presently, it has been recorded from nearly all the European countries and from several countries of the Mediterranean area.

Until the end of the eighties, only a low number of aphid species were identified as PPV vectors. The species identified as vectors were mainly those able to colonize fruit crops hosts of the virus. However, a number of common aphid species were never tested. We carried out transmission tests with 40 species commonly found in our orchard area. 14 of them transmitted PPV in our conditions. Thus, PPV does not seem to differ from other nonpersistently transmitted viruses by its in range of aphid vectors.

The percentage of infectious alatae aphids was monitored within a highly infected orchard. An estimate of 1 infected aphid out of 1000 was obtained. This value was 10 times less than that was estimated with other nonpersistently transmitted viruses in annual crops. PPV transmission rates by *Myzus persicae* were measured under controlled conditions. They were also about 10 times less than those obtained with other nonpersistently transmitted viruses, in agreement with the field data.

The number of aphids alighting on a tree (50,000 to 250,000 on a medium tree) compensates for the low transmission efficiency of PPV. Thus, it seems that PPV differs from other nonpersistently transmitted viruses by its transmission efficiency but not by its range of vectors.

As an applied result, it appears that the elimination of aphids colonizing trees will not be sufficient to decrease the speed of PPV epidemics. On an evolutionary point of view, it seems that an increase of the PPV transmission efficiency is still possible. The virus spread in new areas and its increasing prevalence are favouring factors for such an evolution.

15-026

BIOLOGY OF *CICADULINA* LEAFHOPPERS AND EPIDEMIOLOGY OF MAIZE STREAK VIRUS

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Maize streak geminivirus (MSV) is indigenous in African grasses and is transmitted by *Cicadulina* leafhoppers in a persistent manner. MSV causes sporadic but severe disease outbreaks in maize in sub-Saharan Africa with yield losses approaching 100% on epidemic years. MSV disease epidemics have been attributed to different interacting factors but consensus exists that epidemics are associated closely with the biology of leafhopper vectors. In order to clarify these factors studies have been conducted on the ecology and behavior of *Cicadulina* spp., the incidence and severity of MSV in relation to maize varietal susceptibility, and the role of indigenous grasses as reservoirs of the virus and its vectors. Results of recent studies are presented and the significance of these factors in relation to MSV epidemiology is discussed.

15-025

IMPACT OF BROWN CITRUS APHID (HOMOPTERA: APHIDIDAE) ON DIVERSITY AND DISEASE DYNAMICS OF CITRUS TRISTEZA VIRUS IN FLORIDA

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Establishment of brown citrus aphid (BrCA), *Toxoptera citricida* (Kirkaldy), and severe strains of citrus tristeza virus (CTV) has caused rapid death of millions of citrus trees on sour orange rootstock in South American CTV disease epidemics from the 1930's to the 1970's. In 1993, the aphid became established in Puerto Rico and periodic surveys of CTV incidence there was conducted by serology to determine impact of BrCA on CTV epidemiology and diversity. This is relevant to Florida because in November 1995 the aphid was discovered on dooryard citrus in two counties in south Florida and was declared established in the state.

In 1993, CTV incidence in Puerto Rico was 5% and only 1.4% reacted with MCA13, a severe-strain discriminating monoclonal antibody. By 1995, CTV incidence had increased to 58% and the proportion MCA13 positive reached 26%. Single BrCA transmission rates for two different CTV isolates were 70% and 44%. Although BrCA was caught in low levels (1%) in yellow traps compared to the spirea aphid (56%) and the melon aphid (26%), aphid counts on trees indicated that BrCA was one of the principal colonizers. These data indicated that CTV has increased rapidly after the aphid's establishment in Puerto Rico and that at least two strains of CTV are being spread. A similar increase in CTV spread is now expected in Florida when the aphid becomes established in commercial groves. Because of a high reservoir of CTV decline strains in existing citrus on tolerant rootstock which at present are being spread slowly by indigenous aphid vectors in Florida, a rapid increase in CTV decline is expected as well as an increase in stem pitting CTV. Significant economic damage may result since Florida has 18 to 20 million citrus trees on CTV-sensitive sour orange rootstock and approximately 10.5 million grapefruit trees at risk to stem pitting CTV regardless of rootstock.

15-027

AN EPIDEMIOLOGICAL APPROACH TO MANAGEMENT OF WHITEFLY-BORNE TOMATO INFECTIOUS CHLOROSIS VIRUS

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Tomato production in southern California was threatened in 1993 by tremendous population densities of greenhouse whitefly (GHWF) *Trialeurodes vaporariorum* (Westwood). Additionally, plants in many fields exhibited virus-like symptoms (interveinal chlorosis on the leaves near the base of the plant, subsequently leading to necrosis on the oldest foliage). This new virus tentatively has been named tomato infectious chlorosis virus (TICV).

Epidemiological studies have shown that many plant species in the area contribute to GHWF populations levels, however there is seasonal and temporal variation in the relative contributions of the different species. The majority of GHWF population growth was attributed to crop host species, including strawberry, cucurbits, and tomato. Data suggest that GHWF in this geographic region does not have a tendency for long-distance migration and that infestations of TICV are locally driven. This has a profound impact on the epidemiology of TICV from areas of high incidence to those of low incidence. It also provides an avenue for disease management through vector management.

15-028

WHITEFLIES AND WHITEFLY-BORNE VIRUS EPIDEMICS

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Whitefly-transmitted viruses are a rapidly increasing threat to world agriculture. The years since the mid-1970's until the present have seen the threat of these viruses greatly intensify. The magnitude of the problem with increases in whitefly population densities and the occurrence of whitefly-borne viruses in the tropics and in wide areas of the subtropics, including areas of intensive agricultural production such as the Mediterranean region and southern United States, is largely unexplained. The intensified losses have been attributed to the widespread use of synthetic organic insecticides, resistance to pesticides, enhancement by pesticides, changing climatic conditions, intensified agricultural practices and the international transport of plant material with contaminant populations of whiteflies. Even though some of the diseases induced by these viruses have been known since the early 1900's, and the number of diseases has been estimated at over 100, only relatively few have been characterized, understood, and controlled. Intensified studies on the biological factors and mechanisms of virus distribution including host range, vector transmission, and vector biotypes, are crucial to devising strategies for their control.

15-030

VIRUS/VECTOR/PLANT RELATIONS IN THE EPIDEMIOLOGY OF TOSPOVIRUSES

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A emergence of tospoviruses have been experienced almost worldwide in the last ten or fifteen years. This was accompanied with a revival of research on these viruses in several countries. Much progress have been made on the virus-vector relationships. It has been known for years that the larvae were able to acquire the virus, whereas the transmission was the privilege of adults.

Using antisera to structural and nonstructural proteins viral and nonviral proteins, it could unequivocally be established that the virus replicates in the vector. Transmission studies showed that the virus can be acquired and transmitted in periods as short as 15 min, whereas the AAP₅₀ and IAP₅₀ range between one and two hours depending on the host. In between acquisition and transmission a latent period occurs which, depending on the temperature, ranges between 90 and 180 hours. Second instar larvae proved to be efficient transmitters, when newly born larvae were allowed to feed on infected plants. Approximately, 70 percent of the thrips becoming transmitters does so as larvae. Analyses of the acquisition of virus has showed that only L1 larvae has the unique capacity to acquire virus.

These virus/vector relations, especially the short period required to transmit will determine the dynamics of virus spread in the field. In addition, the random distribution of infected plants, the absence of any effect of the control of thrips on the progress of the disease and a stimulation of the spread by application of repellents shows that a continuous immigration of viruliferous thrips into crops is another factor of importance in the spread of these viruses.

15-029

UV-ABSORBING PLASTIC SHEETS IMPAIR THE EPIDEMICS OF INSECTS VECTORED VIRUS DISEASES

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Field experiments confirmed that crops grown in "walk in" tunnels covered with UV-absorbing plastic sheets were highly protected from infestation with whiteflies, *Bemisia tabaci*, thrips, *Frankliniella occidentalis* and aphids, *Aphis gossypii*. Protection against *B. tabaci*, was associated with a dramatic reduction in the spread of the whitefly-borne geminivirus tomato yellow leaf curl (TYLCV) and with a pronounced delay in plant infection and disease severity. Two month after transplantation, the average TYLCV disease incidence under three different UV-absorbing plastic sheets was 50, 30, and 20% compared with 93% under UV-nonabsorbing polyethylene sheets. In additional experiments, it was shown that the UV-absorbing sheets were also highly effective in the protection of cucumbers against *F. occidentalis* and *A. gossypii*. The association of these phenomena with the "UV vision" of insects will be discussed.

15-031

ANALYSIS OF THE DYNAMICS OF VIRUS TRANSMISSION BY VECTORS

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Plant virus diseases have generally been classified according to transmission characteristics and the behaviour of the virus in the vector. For insect, mainly Homopteran, vectors four categories have usually been recognised: non-persistent, semi-persistent, persistent-circulative; and persistent-propagative. Only in the last category does the virus multiply in the vector and in some cases there can be transovarial transmission to offspring.

A general epidemiological model has been developed which can deal with the four categories of virus transmission by vectors. Additionally host mortality due to disease, and vector birth, death and migration rates can be included. In cases where there is a constant population size, then a full qualitative analysis of the dynamics is obtained. A basic reproductive number is derived which describes both plant-to-vector and vector-to-plant transmission.

Vector activity and turn-over rate are important factors determining whether the disease will persist in the host and vector populations. It is also possible to deal with both immigration and emigration explicitly but interpretation of the qualitative results is difficult except using graphical techniques. The analysis is used to compare the effectiveness of vector control, eg. by insecticides or vegetation management, with sanitation measures involving the removal and destruction of diseased plants.

15-032

ESTIMATING OVERWINTERING POTENTIAL FLOWER THIRPS FROM DIFFERENT GEOGRAPHICAL AREAS
K. Walters

ABSTRACT NOT RECEIVED

15-033

BIOTYPIC VARIATION IN WESTERN FLOWER THIRPS IN RELATION TO DURABILITY OF HOST PLANT RESISTANCE IN CUCUMBER

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Western flower thrips, *Frankliniella occidentalis* (Pergande), is a serious pest on many ornamental and vegetable crops. Development of resistant cultivars is one of the strategies to control this insect. Biotypes of insect pests, however, may overcome the protective properties of such cultivars. The study of biotypic variation is therefore of prime importance. Variation in performance in a worldwide collection of western flower thrips was studied on susceptible and partial resistant cucumber genotypes. Leaf disc assays indicated that some strains from New Zealand and USA were less aggressive than the reference strain from The Netherlands. None of the strains tested was more aggressive than the reference strain. Selection experiments are performed in order to study the possibility of adaptation of thrips to partial resistant cucumber plants. First results indicate a slightly higher reproduction and lower mortality of a selection line of thrips on partial resistant cucumber after a few generations. Results are discussed in relation to durability of host plant resistance to western flower thrips.

15-034

TOSPOVIRUS TRANSMISSION BY WESTERN FLOWER THIRPS FROM DIFFERENT GEOGRAPHIC REGIONS

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The tospovirus/western flower thrips (WFT) complex is of worldwide concern to the greenhouse industry as well as to crops in the open field. In view of the worldwide spread of WFT, its ability to infest many plant species and its occurrence in different biological niches, diversity at different levels may exist between various populations. A number of WFT populations sampled in Italy (bean), France (cucumber), Israel (mango, strawberry), USA (chrysanthemum 2x, lemon), New Zealand (egg plant), and the Netherlands (chrysanthemum, cucumber, bean) were compared for the frequency with which they transmitted two tospoviruses, tomato spotted wilt virus (TSWV) and *Impatiens* necrotic spot virus (INSV), using the petunia leaf disk assay. All populations were able to transmit these tospoviruses, although differences in efficiencies were found. Thrips originating from France, Israel (strawberry), New Zealand and the Netherlands transmitted twice more frequently than populations from USA, but twice less frequently than the thrips from Israel (mango) and Italy (bean). These results provide more insight in the evolution of the various tospovirus species and WFT thrips populations.

15-035

SYMBIOTIC BACTERIA IN WESTERN FLOWER THIRPS AND VARIATION BETWEEN DIFFERENT THIRPS STRAINS

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Symbiotic microorganisms are a widespread in phytophagous insect species. They are often associated with the digestive system. These symbionts are important in the ecology of the insect host: e.g. they supply essential nutrients or degrade toxic plant compounds. We study the role of bacterial symbionts in host plant adaptation in Western Flower Thrips.

Western Flower Thrips (*Frankliniella occidentalis*) is a world wide insect pest on a large number of greenhouse crops. We investigated the composition of gut bacteria in thrips populations from different host plants. Symbiotic bacteria isolated from the insects were cultured outside the thrips. Analysis of the 16S rDNA sequences made clear that these bacteria belong to the family Enterobacteriaceae, closely related to *Escherichia coli*. Detailed screening with RAPD markers showed that these bacteria were associated with the thrips strains over generations and that each thrips strain contained one type of symbionts. Genetic variation in and between thrips strains was analysed using RAPD markers, RFLP markers and sequencing of the ITS 1 region of rRNA genes.

15-036

HOW AND WHY DO THRIPS TRANSMIT TOSPOVIRUSES: A CELLULAR APPROACH TO UNDERSTANDING

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The tospoviruses are important emerging phytopathogens causing severe epidemics worldwide. Among the 5,000 or so known thrips species, only seven transmit tospoviruses. The thrips/tospovirus relationship is unusual in that transmission occurs only when first instar larvae acquire the virus. There is also apparent specificity between tospovirus species and their thrips vectors. The known vector species represent two of the largest genera of the Thripidae. Although the species involved do not form closely related groups phylogenetically, they do share important ecological characteristics. Recently, the status of several vector species has apparently changed dramatically, i.e. *Thrips tabaci*, once the most important vector species worldwide apparently no longer transmits contemporary isolates and *Thrips palmi*, formerly thought to be a nonvector, transmits at least three important tospoviruses. Close scrutiny of thrips/virus interactions suggests that evolution of tospoviruses and/or selection pressures on both the viruses and vectors contributes to changing vector specificities. On a cellular level evidence from electron microscope studies indicate that acquisition of tomato spotted wilt tospovirus (TSWV) is receptor mediated. The location of the viral glycoproteins (gps) in the virion envelope, presence of a RGD-motif in the gp2 and the absence of this motif in nontransmissible TSWV deletion mutants support the hypothesis that TSWV gps serve as viral attachment proteins. Analyses to identify the counterpart cellular receptor site in thrips, reveal an approximately 50 kD thrips protein that binds both TSWV gps, but not TSWV nucleocapsid protein or the nonstructural protein encoded by the TSWV S RNA. Similar preparations from leafhoppers, aphids and non-vector thrips species did not bind any TSWV proteins. Furthermore, anti-idiotypic antibodies that mimic TSWV gps detected the 50 kD thrips protein. These data suggest the 50 kD thrips protein is a component of the cellular receptor site for TSWV attachment proteins and may play a critical role in virus acquisition. The relevance of these findings to possible mechanisms underlying emergence of tospovirus/thrips relationships and vector specificity will be discussed.

15-038

THE RELATION BETWEEN SAPROPHAGY AND PHYTOPHAGY IN THRIPS

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Some Thysanoptera species in the families Phlaeothripidae and Thripidae, living in leaf litter and vegetative parts of forest plants, were studied relating to their feeding activity. Differences in both the feeding apparatus and gut structure are reported and discussed. Biological data related to field populations are also provided.

15-037

THRIPS' (THYSANOPTERA) RESPONSES TO VARIABLE ABIOTIC FACTORS

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Thrips are widespread throughout the world. The majority of species are tropical, many are temperate, and only a few species extend into Arctic regions. Many species of tropical and subtropical origin have become widely established in glasshouses in temperate regions where they may cause considerable economic damage. For the past two decades, especially the subtropical *Frankliniella occidentalis* and the tropical *Thrips palmi* have expanded their distribution area by infesting glasshouse crops in colder regions.

The interest in obtaining a thorough knowledge on the biology of economical important thrips species has increased as control of these species with pesticides has become difficult or undesired. Detailed knowledge on thrips' biology under different environmental conditions is essential to, e.g., pest risk assessments, timing of control measures, or utilisation of alternative control measures.

Knowledge on tolerance to unfavourable abiotic conditions in thrips infesting glasshouses is essential to illustrate the species' ability to overwinter under ambient conditions. Such knowledge may also reveal possibilities for manipulating glasshouse environments to increase mortalities or to slow down developmental rates to such an extent that integrated control will be more successful.

The present presentation gives a mini-review on thrips' responses to selected variable abiotic factors with emphasis on economical important species.

15-039

ONTOGENIC DEVELOPMENT OF THE DIGESTIVE SYSTEMS OF THRIPS

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ABSTRACT NOT RECEIVED

15-040

PROBLEMS AND PROSPECTS OF MANAGING *BEMISIA* IN PAKISTAN

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The cotton whitefly, *Bemisia tabaci*, is now posing a serious threat to cotton in Pakistan due to both its direct damage and acting as a vector of leaf curl virus. In 1992, 1993 and 1995, it was the major cause of yield reductions in cotton. Its outbreaks during the nineties were characterised by poor control by many recommended insecticides. Monitoring of whitefly populations from 1992 through 1995 reveal that a high level of resistance has developed to organophosphates like dimethoate, methamidophos and monocrotophos, and pyrethroids like cypermethrin and deltamethrin. In this situation, control practices for whitefly and other cotton pests need to be modified with special emphasis on the proper application techniques and non-chemical control methods to reduce whitefly populations and conserve natural enemies. A rotation of still effective insecticides including new chemicals having different modes of action, along with other IPM tactics, is recommended to combat whitefly

15-042

LABORATORY AND GREEN-HOUSE STUDIES OF INSECTICIDE RESISTANCE IN *BEMISIA TABACI* GENNADIUS (HOMOPTERA: ALEYRODIDAE)

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Many populations of *B. tabaci* found in glass and plastic greenhouses in Europe have been exposed to heavy selection pressure from a range of insecticides, including the established compounds such as organophosphates and pyrethroids as well as the newer insecticides such as buprofezin and imidacloprid. Examples of populations resistant and susceptible to these insecticides were examined in the laboratory and in the field, and experiments conducted to demonstrate the impact of the levels and frequencies of the resistance genes on the field performance of the insecticides. In some populations buprofezin resistance was unstable, although re-selection was rapid. This instability was correlated with increased development times compared to susceptible strains. *In situ* selection for resistance plus passive transport of resistance genes to other regions highlights the difficulties of controlling this pest and suggest that insecticide resistance management strategies for *B. tabaci* need to be considered within an international perspective.

15-041

LOCALIZED MIGRATION BY *BEMISIA*

Laboratory populations of the sweet potato whitefly, *Bemisia tabaci*, have been shown to consist of both migratory and trivial flying morphs. The behavior of these forms to be examined under field conditions. Insects were marked in a field of cantaloupes using fluorescent dust. During the first growing season, passive traps, used to collect living whiteflies, were placed along 16 transects radiating out from the field to a distance of up to 1.0 km. Wind out of the northeast consistently carried migrating whiteflies to traps along placed along transects in the southwestern quadrant because cold air drainages dictate wind. For this reason, during the second season traps were laid out in a grid extending 2.7 km to the southwest of the marked field. If dispersal was solely was entirely passive or wind directed, patterns could be described using a diffusion model. Statistical examination of the data, however, demonstrate that the distribution on all days was patchy. Traps in the immediate vicinity of the marked field caught more whiteflies than the daily median. Large numbers were also collected from around the periphery of the grid. Whiteflies were far less prevalent in the center. As a result, the distribution of captured whiteflies can be described as bimodal. These patterns confirm behavior observed in the laboratory, i.e., a portion of the population are trivial fliers that do not engage in migration and are consequently captured in traps near the field, and a portion initially ignore vegetative cues and fly for a period of time before landing in distant traps so that they comprise the second peak in the model. During both years movement out of the field had an exaggerated directional component as evidenced by highly significant chi-square values produced on 13 of 14 days.

15-043

BEMISIA RESISTANCE MONITORING AND PREVENTION IN THE FIELD

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Whiteflies threaten the economic viability of crop production in many areas of the world. They are inherently difficult to control with pesticides and have rapidly developed resistance to virtually all chemicals used for their control. Whitefly resistance problems have constituted the classical resistance treadmill in which pest resistance has caused increased rates and numbers of applications of insecticides and this in-turn has resulted in more rapid loss of effectiveness of the remaining chemical controls. Overcoming this resistance treadmill in whiteflies constitutes the objectives of collaborative research programs based in England, Israel, California and Arizona. With examples from the aforementioned programs, I will overview current efforts to: 1) reduce overall insecticide use through implementation of alternatives to conventional pesticides, 2) identify and obtain registration of 'softer' insecticides, such as insect growth regulators, 3) formulate, implement on an area-wide basis and evaluate provisional resistance management strategies, and 4) establish and maintain resistance monitoring programs that continually evaluate and improve resistance management strategies targeting whiteflies.

15-044

THE IMPACT OF PREDATORS ON *BEMISIA* POPULATIONS.D. Gerling¹, S. Naranjo², and Moshe Guershon¹¹Department of Zoology, the George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel - ²Western Cotton Research Laboratory, USDA, ARS, USA.

Numerous predatory arthropods are able to prey on *Bemisia*. The most prominent species belong to the Heteroptera, Neuroptera and Coleoptera. However, it is difficult to assess the impact of the predators on *Bemisia* populations for a number of reasons. Many are general predators that may prefer other prey, and are not well adapted to feed on *Bemisia* either nutritionally or behaviorally. The predators are mobile, and often they and evidence of their activity are hard to find.

Several techniques have been used to determine which predators have the largest impact on the whitefly populations. These include nutritional studies, whole plant samplings, *in situ* field observations, and immunoanalysis using monoclonal antibodies. So far these studies have shown that some species of Coleoptera, and Heteroptera appear to have the greatest impact on *Bemisia* populations.

15-046

THE GEMINIVIRUS CAPSID PROTEIN: EVIDENCE FOR A DIRECT ROLE IN WHITEFLY-MEDIATED VIRUS TRANSMISSION
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Whitefly-transmitted geminiviruses, or subgroup III Geminiviridae, are important viral pathogens of crops cultivated in dry subtropical/tropical locales, worldwide. A single species of whitefly, *Bemisia tabaci* (Genn.), is the universal vector of the subgroup III geminiviruses, based on its ability to experimentally transmit viruses indigenous to the Eastern and Western Hemispheres (Bedford et al., 1994). This indicates highly specific virus-vector interactions, and implicates a conserved viral gene in the transmission process. The capsid protein (CP) gene is the most highly conserved gene of the subgroup III geminiviruses. Alignment of the predicted CP amino acid sequences of representative subgroup III viruses reveals 80-96% sequence identity (Rosell & Brown, 1993). In this model, the viral CP, assembled into nucleoprotein particles, interact directly with sites in the whitefly vector during the uptake, acquisition, and/or transmission processes. PCR-based monitoring of squash leaf curl virus ingested by the whitefly vector, *B. tabaci* and a non-vector whitefly, *Trialeurodes vaporariorum* (West.) indicates the presence of virus in insect extracts and honeydew of both whitefly species, and virus is detectable in saliva excreted by the whitefly vector, but not in saliva from the non-vector whitefly; this is the first direct evidence of transmissible virus in whitefly vector salivary excretions (Rosell & Brown, 1996). Further, replacement of the CP of African cassava mosaic virus (ACMV) by the analogous gene of the leafhopper-transmitted beet curly top virus (BCTV) (subgroup II) yields ACMV DNA encapsidated in BCTV coat protein, and rendered the hybrid virus leafhopper-transmissible (Briddon et al., 1990). CP mutants that do not make coat protein are not vector transmissible (Azzam et al., 1994; Brown et al., 1996). Exchange of the CP of the whitefly-transmissible SqLCV with that of the non whitefly-transmissible abutilon mosaic virus (AbMV) abolishes vector-transmissibility, and replacement of AbMV CP with SqLCV AR1 gene yields a vector-transmissible hybrid (Brown, Hartitz, Rosell & Bisaro, 1996). Collectively, these results demonstrate a direct role for geminiviral capsid protein in whitefly-mediated geminivirus transmission.

15-045

THE IMPACT OF PARASITOIDS ON *BEMISIA* POPULATIONS.

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Biological control programs against whitefly have been very successful relative to programs against other groups of insect pests, and parasitoids have been the natural enemies credited with this success. However, biological control programs directed against *Bemisia* face some unique challenges. Unlike whitefly pests of orchard systems, *Bemisia* has a very wide host range and is generally a pest of annual crops, in which habitat disruption and pesticide use are frequent occurrences. In the large agricultural valleys in the southern U.S. where *Bemisia* is a pest, populations migrate from crop to crop, building up in size as the year progresses.

The following questions will be addressed. What is the potential for parasitoids to control *Bemisia* in a multiple cropping system? Can the use of parasitoids be integrated with current chemical control practices? What is the relative role of native and exotic parasitoids in biological control of *Bemisia*? What are the likely effects of the unusual autoparasitoid life history on biological control of *Bemisia*? Lastly, the current evidence for the reduction of *Bemisia* populations as a result of parasitoid attack will be reviewed.

15-047

BEMISIA TABACI (GENNADIUS) (HEMIPTERA:HOMOPTERA:ALEYRODIDAE), BIOTYPE/HOST/VIRUS INTERACTIONS

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NR4 7UH.

Bemisia tabaci, the tobacco whitefly, has usually been associated with tropical and subtropical crops. It can transmit plant viruses in six different groups including approximately 60 geminiviruses. *B. tabaci* populations from locations worldwide have been characterised by DNA and esterase analysis, size, mating behaviour and in rare cases by larval morphology. Most populations could transmit most geminiviruses from any location. Many populations of *B. tabaci* prefer a narrow range of host plants; some populations are oligo or monophagous although most may eventually adapt to a wider host range. This narrow host range of most populations (non-B biotypes) may restrain the spread of whitefly-transmitted viruses.

The most polyphagous strain of *B. tabaci*, (the "B" biotype or "Silverleaf whitefly"), has evolved and been spread around the world through the movement of ornamental plants, produce and cut flowers. Its wide host range has increased the potential for whitefly-transmitted viruses to infect new host plants and crop species. Its increased fecundity and ability to induce phytotoxic damage increased its pest status.

Transmission of geminiviruses is a complex of interactions including those between the virion, virus replication, viral movement proteins and the feeding behaviour of the vector. Clones and mutants of insect transmissible and non-transmissible isolates of African cassava mosaic virus (ACMV), show that both cell to cell movement proteins and coat proteins play a key role in acquisition and transmission processes respectively.

15-048

WHITEFLY PROBLEMS AND RESEARCH IN LATIN AMERICA

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Endemic and introduced whitefly species have widely colonized tropical to temperated regions of the subcontinent. With few local exceptions, only the 'greenhouse whitefly' (*Trialeurodes vaporariorum*), adapted to (sub-)tropical highland conditions, and specially the *Bemisia tabaci* complex have produced consistently severe yield losses in several economic relevant crops, the latter mainly in arid lowlands. Besides through causing direct damage by sucking, indirect effects like honeydew production, phytotoxic disorders and/or the transmission of whitefly-specific geminiviruses (WFTG) have been limiting an increasing amount of crops in numerous regions.

WFTGs have been appearing in many regions and distinct crops like beans, peppers, cotton and cucurbits causing periodically (1970-90) economic losses in Brazil, Argentina, Central America and the Caribbean, being responsible with regional differences endemic *B. tabaci* biotypes (e.g. the 'A' biotype). Sudden outbreaks (1987/88) and severe attacks to formerly not reported hosts (e.g. tomatoes, pepper, cucurbits and cabbage) in countries of the Caribbean basin pointed to a recent introduction of the Old World 'B-biotype'. Linked to this so called 'silverleaf whitefly' (syn. *B. argentifolii*), new phytotoxic disorders appeared, e.g. the silvering of cucurbit leaves and uneven ripening of tomato fruits. Heavy WFTG infestations produced dramatic yield losses in tomato and fields being involved New World WFTGs in South and Central America besides to the recently introduced 'tomato yellow leaf curl virus' (TYLCV-Is) in some Caribbean islands. The continuous spread of the 'B-biotype' all over the subcontinent has recently been reported causing problems in Ecuador, Peru and other countries. Due to severe economic losses and an important reduction of crop areas, national research and extension activities have been increased under international co-operation in most of the countries. Research efforts have been focused on an IPM approach including monitoring, improved chemical control (incl. resistance management, selective compounds), application techniques, testing tolerant genetic materials, new (cultural) strategies for 'small farmers' incl. seedbed protection (e.g. covers, living barreers, sticky traps), natural and classical biocontrol (incl. parasitoids, predators and entomopathogens). During the last two seasons tomato growers of different regions have been able to reduce yield loss substantially due to improved extension activities, legal measures, chemical and fitogenetic control practices as well as climatic factors.

15-050

GENETIC COMPATIBILITY AMONG DIAPAUSING RED, NON-DIAPAUSING RED AND DIAPAUSING GREEN FORMS IN *TETRANYCHUS URTICAE*

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The occurrence of diapause and reproductive compatibility were studied among three different forms of the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), found in Japan. The three forms were the diapausing red (DR) form (first described here), the non-diapausing red (NDR) form (formerly called *T. cinnabarinus*) and the green (G) form. Almost all DR and G females entered diapause, whereas females from NDR completely lacked diapause.

In intra-population crosses, all populations produced both female and male progeny. Inter-population crosses within the same forms also resulted in the production of both sexes, suggesting that these populations were compatible. However, no fertile F₁ females were produced in crosses between DR and either NDR or G, indicating that the former was reproductively incompatible with the latter two. On the other hand, NDR mites produced a smaller number of fertile F₁ and F₂ females when mated with G mites, showing that gene exchange between them is possible. Thus, it seems that NDR and G are conspecific, although they show severe genetic incompatibility.

15-049

RECENT ADVANCES IN AGRICULTURAL ACAROLOGY

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Much work was performed on agricultural acarology after the Second World War and until the mid-1980s. Most publications were devoted to systematics and field research by agronomists to provide a rapid solution to the problems raised by outbreaks of phytophagous mites on crops. Research in this field has necessarily become increasingly academic and calls on all the disciplines developed in recent years:

-the proposal of mathematical models thanks to progress in data processing; such models are used both for the analysis of population dynamics and the drawing up of spraying schedules;

-analyses of the various aspects of the behaviour of the pests and that of predators;

-studies of population genetics first using classic methods, then based on isoenzyme variability and finally involving a range of molecular biology techniques. The aims are the search for genome markers, better definition of biological entities, the determination of phylogenetic relations, analysis of exchange between populations and attempts at genetic transformation to increase the potential of predators.

15-051

USEFULNESS OF DNA SEQUENCE INFORMATION IN THE ANALYSIS OF TETRANYCHID INTRASPECIFIC VARIABILITY

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Ribosomal and mitochondrial DNA variation were used to study intraspecific divergence in three tetranychid species. Mites originating from 29 localities distributed world-wide (19 for *Tetranychus urticae* Koch, 4 for *Mononychellus progresivus* Doreste and 6 for *Amphitetranynchus viennensis* (Zacher)) were analyzed by polymerase chain reaction amplification and direct sequencing of both the internal spacer ITS2 and part of the cytochrome oxydase I gene (COI).

Mitochondrial COI variations were detected between localities in the three spider mites. ITS2 sequences were virtually identical among *T. urticae* samples whereas there were appreciable differences in the two other species. Ecological features such as feeding specificity and geographic distribution may explain the contrasted patterns of molecular divergence. The relevance of nucleotide sequences in assessing intraspecific variability in spider mites is discussed.

15-052

PERSPECTIVE ON ERIOPHYOID MITE RESEARCH

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This lecture deals briefly with the current status of the knowledge on Eriophyoidea (Acari Prostigmata), which are obligatory phytophagous Mites. Some points need attention for developing guidelines in a future progress regarding mainly a faunistic and systematic point of view, morphology, anatomy, biology, reproductive strategies, direct and indirect injuries, tritrophic inter-relationship, natural enemies, biological weed control, etc.

A few aspects, for instance, that could be promoted, are:

- 1) to organize a useful instrument for the researchers consisting in a database (i.e. on CD) containing information on the original description and illustration, biogeography, habit, hosts, references, etc., that may be easily and quickly looked up;
- 2) to more deeply investigate some anatomical aspects for which there are scarce information such as the nervous system, sensory organs, gonads, differences between deutogyne and protogyne, male and female, immature stages and adult.
- 3) to individuate and improve rearing techniques for studying reproduction, development, behaviour and life-history strategies;
- 4) to ascertain the salivary compounds involved in biochemical mechanisms inducing galls, rusting, edge-rolling, deformations, and other alterations on plants;
- 5) to look into the relationship between eriophyoids and plant pathogens (virus and mycoplasma) for carrying out the transmission mechanisms;
- 6) to continue in verifying the role and the importance that Eriophyoid populations play in the alimentary chains.
- 7) to establish appropriate criteria for identifying and selecting promising candidates in weed control and estimating their potential effectiveness.

15-054

THE FEEDING EXPERIENCE OF POLYPHAGOUS SPIDER MITE (*TETRANYCHUS URTICAE* KOCH) THAT ALTERS THE ACCEPTABILITY OF UNEXPERIENCED HOST PLANTS

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Adult females of the phytophagous mite, *T. urticae*, escape from unfavorable plant resources. We experimentally introduced the mites onto leaf disks of their potential hosts. Host qualities were ranked in terms of the fecundity the mites exhibited on them. The mites tended to escape from any hosts when they had previously experienced hosts of higher rank, whereas they tended to accept any hosts when they had experienced hosts of lower rank. The result suggested that the mites accurately evaluated unexperienced plant resources on the basis of their earlier feeding experiences. The characteristics of host acceptability of the mites will be discussed with respect to their host range in the field.

15-053

DISTRIBUTION, BIOLOGY, PEST STATUS AND CONTROL STRATEGIES OF THE MOST IMPORTANT PHYTOPHAGOUS MITES OF TEMPERATE FRUITS IN ITALY

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The economic importance of phytophagous mites in Italy has greatly increased after the second world war corresponding with dramatic changes in agricultural practices. Mite outbreaks appeared to be more frequent in orchards, vineyards and protected crops and chemical control represented the main response to this problem until the late 70's. Mite resistance to broad-spectrum pesticides was initially contrasted by a rapid succession of acaricides. Failures of chemical control and the occurrence of secondary pests, due to the side-effects of pesticides, allowed for the spread of the IPM approach in several areas.

The most serious pests of temperate fruits in Italy belong, in particular, to the families Tetranychidae and Eriophyidae while the importance of other groups (e.g. Tenuipalpidae and Tarsonemidae) is lower. Among the Tetranychidae, some species are widespread and economically important in orchards and vineyards (e.g. *Panonychus ulmi* and *Tetranychus urticae*), others (e.g. *Eotetranychus carpini*) are distributed more locally or occur in neglected or unsprayed orchards (e.g. *Bryobia rubrioculus*, *T. viennensis*). The Eriophyidae can be injurious especially on apple (e.g. *Aculus schlechtendali*), pear (e.g. *Epirimerus pyri*), peach (e.g. *Aculus fockeui*) and grapevine (e.g. *Calepitrimerus vitis*).

Some notes on the biology, behaviour and economic importance of the most serious pests of temperate fruits in Italy are briefly reported with a summary of recent progress in biological and integrated control strategies on apple, pear, peach and grapevine.

15-055

TRANSMISSION BIOLOGY OF *ERIOPHYES INSIDIOSUS*-VECTORED PEACH MOSAIC VIRUS

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Peach mosaic is an infectious disease that impacts peach production in California and Mexico. Our studies have shown that the causal agent is a closterovirus-like particle. Experiments to elucidate the transmission biology have shown that transmission efficiency varies with the specific buds from which inoculative mites are taken. In two sets of experiments we found that transmission efficiency of single mites was 15.4% whereas transmission by 5 and 10 mites was 2.7%. We also have determined the minimum inoculation access period to be as low as 6 hr. Experiments to test the minimum acquisition access period and latent period are in progress. Information gained through these experiments not only will further our understanding of mite-vector pathogens but may prove useful in designing management strategies.

15-056

A CASE OF SIBLING SPECIES IN *TYPHLODROMALUS* (ACARI: PHYTOSEIIDAE)

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Phytoseiid mites identified as *Typhlodromalus aripo* DeLeon have been commonly reported in South America on cassava plants (*Manihot esculenta* Crantz) and several kinds of weeds. The fact that on the former plants those phytoseiids are found almost exclusively in the growing tips whereas on the latter they are found on fully grown leaves prompted the investigation of a possible case of sibling species. Laboratory crossing studies were conducted with specimens collected from cassava and from *Baccharis* sp.. They indicated that matings occurred between individuals of the same or different substrates. Oviposition was only observed when females were mated, and almost all females oviposited when mated with males from the same substrate, producing more than 85% of viable eggs. No eggs were produced by females from cassava crossed with males from *Baccharis*. Most females from *Baccharis* oviposited when crossed with males from cassava; however, resulting F1 females did not oviposit when crossed with males from either substrate or F1 males. Morphological evaluation of mites used in the crossing studies failed to show any reliable differences. Populations of this mite group collected from cassava in Brazil have been mass produced for releases in cassava fields in Africa for the control of the cassava green mite, *Mononychellus tanajoa* (Bondar). A re-evaluation of the correct identity of those populations is warranted in light of present knowledge.

15-058

PHYLOGENETIC AFFILIATIONS OF FULGOROMORPHA (HEMIPTERA: ARCHAEORRHYNCHA) INFERRED FROM 18S rDNA NUCLEOTIDE SEQUENCES

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Evolutionary affiliations of major groups of insects in the order Hemiptera (*sensu lato*) have been debated for more than a century. Based on various morphological features of wings, mouthparts, head, antennae, genitalia and alimentary canal, a number of conflicting phylogenetic scenarios have been proposed. Shared characters of aristate antennae and tymbals have been used to place fulgoromorphans and cicadomorphans in the traditional suborder, Auchenorrhyncha. A thorough examination of both fossil and extant hemipteran taxa reveal that these "auchenorrhynchan synapomorphies" could likely be the result of convergence. Alternatively, tree topologies of major hemipteran lineages based upon homologous nucleotide sites of the gene encoding 18S rRNA (18S rDNA) suggest: 1) there are at least four main monophyletic lineages in extant Hemiptera (sternorrhynchans, cicadomorphans, fulgoromorphans and coleorrhynchans), 2) Sternorrhyncha is sister to all other Hemiptera (*i.e.*, Euhemiptera *sensu* Zrzavy), 3) there is little or no support for a monophyletic Auchenorrhyncha, and 4) radiation of these four major lineages occurred rapidly from a common ancestor. A renaming of hemipteran suborders to better reflect natural, evolutionary affiliations has been proposed by Sorensen *et al.* The best supported topology based on molecular data indicates that fulgoromorphans are sister to a clade consisting of true bugs and peloridiids. This monophyletic clade has been named Neohemiptera.

18S rDNA nucleotide sequences also provide possible insights into family-level evolutionary affiliations among fulgoromorphans. A molecular based phylogeny supports a basal elade of cixiids and delphacids. However, the enigmatic tettigometrids appear to occupy a distal clade with that of tropiduchids and flatids.

15-057

VOLATILE PLANT COMPOUNDS AS ANTIFEEDANTS FOR A PHYTOPHAGOUS MITE

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In the plant/mite interaction *Halotydeus destructor* (Tucker) - *Trifolium subterraneum* (L.), numbers of mites are lower and they feed less on cotyledons of resistant varieties, than of susceptible varieties, during a three hour test period. Artificially damaged cotyledons release several volatile metabolites, including 1-octen-3-one, arising from lipid peroxidation. The levels of 1-octen-3-one in different *T. subterraneum* varieties are negatively correlated with levels of feeding damage to the varieties. In a bioassay using *H. destructor*, the EC₅₀ for 1-octen-3-one deterrent activity is 50ppm, while at 1000 ppm all mites are repelled from feeding. The 1-octen-3-one is produced in a time-dependent manner from artificially damaged cotyledons. The wound induced C₈ compound, 1-octen-3-one, is considered to play a role in the deterrence of cotyledons of some resistant subclover varieties.

When foraging, *H. destructor* are very restless, running, palpating (tapping the surface), probing (pausing to apply mouthparts to the plant surface), and feeding (stationary, with legs braced on the plant surface). A hole is made in the epidermal surface, through which the cell contents are sucked when feeding. Mite feeding elicits production of the volatile compounds from peroxidised lipids in damaged cells. On non-preferred hosts, the mites palpate and probe, but seldom settle down to feed. We suggest that mites face the highest concentrations of the antifeedant volatiles, in either liquid or vapour form, at the plant surface after probing, and are deterred from further feeding. Identification of resistance mechanisms is helping in a program to select and breed annual pasture legumes with resistance to *H. destructor* feeding.

15-059

INFERENCE OF PHYLOGENETIC AFFILIATIONS OF FULGOROMORPHA (HEMIPTERA, ARCHAEORRHYNCHA) BASED UPON MORPHOLOGY

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Hypotheses in phylogenetic relationships of Tettigometridae within Fulgoromorpha families have never reached a full agreement. Most authors regard the Tettigometridae as the sister-group of all other fulgoromorph taxa (Asche, 1988; Emljanov, 1990; Wilson *et al.*, 1994) and this hypothesis has been used in studying evolutionary patterns (Denno & Perfect, 1994, and contributors in it). Accuracy of these studies is directly related to the quality of phylogenetic assumptions employed. However, there are at least two other phylogenetic hypotheses based on morphological data.

The possibility that Tettigometridae represents a relatively distal lineage of Fulgoromorpha was proposed by Bourgoin (1985) based on a cladistic assessment. In this study the Tettigometridae were grouped in a lineage with the Flatidae and the Tropiduchidae but their relationships were not specified. Since then, Bourgoin has firmly established the monophyly of the tettigometrids (1987) and reinforced his contention in several papers dealing with new characters (1986, 1993, Bourgoin & Deiss, 1994), and with biogeographical arguments (1988, in press) Yang & Fang (1993), based on a phenetic assessment, regarded the Fulgoromorpha as a paraphyletic taxon in that the Tettigometridae were more closely related to the Cicadellidae than to other families of fulgoromorphs.

These varying phylogenetic hypotheses raise two basic questions: 1) Are the Fulgoromorpha a monophyletic taxon ? and, 2) What is the sister group to the Tettigometridae ?

This contribution presents a new cladistic analysis based on morphological characters and concludes in favor of the monophyly of the Fulgoromorpha and the non basal position of the Tettigometridae. This phylogeny is compared to that based on DNA and the position of Fulgoromorpha within Hemiptera is also discussed.

15-060

THE PHYLOGENETIC RELATIONSHIPS OF FULGOROMORPHA BY DNA SEQUENCE ANALYSES

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Phylogenetic reconstruction of the family relationships within Fulgoromorpha based on sequence information was conducted using DNA sequences of two genes. The sequence comparisons of 420 bases of the 3' end of the 16s rRNA sequences from 15 taxa of 11 families and those of 414 bases of the 5' end of the cytochrome b genes from 10 taxa of 7 families reveal general congruence. Both genes suggested that Meenoplidae is a basal lineage among those taxa sampled and that Issidae is a non-monophyletic group. The results of 16s rRNA sequence did not group Dictyopharidae with Fulgoridae, as suggested by morphological characters, but was closer to Derbidae.

15-061

THE NYMPHAL ANTENNAE OF AUCHENORRHYNCHA (HOMOPTERA): A PHYLOGENETIC TEST

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The antenna of first to fifth instar nymphs of Peloridiomorpha and Auchenorrhyncha (Archaeorrhyncha + Clypeorrhyncha *sensu* Sorensen) were examined. The developmental arrests at the widest part of the basiflagellum in the Membracoidea and Fulgoroidea were observed and treated as a synapomorph of the two superfamilies. The polymerized antennae of Cercopoidea and Fulgoroidea are homoplasious. Mapping the character of number of nymphal antennal segments upon four given phylogenetic trees of Hemiptera using the program MacClade ver. 3.01 indicates that 3-segmented antennae of fifth instar nymph is the synapomorph of Euhemiptera and was derived from the 7-segmented antennae, which is the most primitive state within Hemiptera. While 3-segmented antennae is the primitive state of Auchenorrhyncha, the 9-segmented antennae in fifth instar nymph is a synapomorph for the (Cicadoidea + Cercopoidea). The hypothesized ground plan of the fifth instar nymphal antenna of Auchenorrhyncha *sensu* Zrzavy is 3 segments, and without developmental arrests. Auchenorrhyncha is reconsidered as a monophyletic group according to this specific feature.

15-062

THE REPRODUCTIVE APPARATUS IN CIXIDIA (HOMOPTERA, AUCHENORRHYNCHA, ACHILIDAE)

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Male and female reproductive apparatus of *Cixidia sikaniae* were investigated.

In male, each testis consists of 6 follicles; in living specimens, both vas deferens and seminal vesicles appear orange-coloured; the ejaculatory duct is very long and receives a pair of long and convoluted accessory glands.

In female, each ovary consists of 6 ovarioles with long pedicels; both lateral and median oviducts are short; the latter widens to form a broad chamber; the spermatheca is long and complicated; the bursa copulatrix is broad and bears a sclerified plate.

15-063

EVOLUTIONARY PATTERNS OF HOST PLANT USE BY PLANTHOPPERS (HEMIPTERA: FULGOROIDEA)

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Planthopper host plant associations were surveyed for 17 of the 19 families to determine if there was evidence for parallel diversification of planthoppers and their host plants. Parallel evolution between planthoppers and host plant taxa was not found, however, there was evidence for phylogenetic patterns in feeding location. In one family, the Delphacidae, weak evidence for parallel diversification with monocot taxa was found as well as a strong tendency toward monophagy. Departures from this pattern suggest that host shifts may be influenced by habitat fidelity as exemplified by the host associations of Pacific Island faunas.

15-064

THE ROLE OF TAXONOMY AND BIOLOGY IN PATTERNS OF PEST AND VECTOR DISTRIBUTION WITHIN THE FULGOROMORPHA (HEMIPTERA)

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The distribution of pest species and the vectors of plant disease is not evenly spread within the planthopper families (Hemiptera; Fulgoromorpha). For instance, the Delphacidae contains the majority of pest and vector species, while some large, species-rich families contain no known pest species.

Pest species distribution within and between families are analysed. Reasons for the observed patterns of pest and vector species, related to both taxonomy and host preferences are discussed. In the majority of cases, if not all cases, the interaction of human agricultural use of plant species and insect host preferences are the main determinants.

15-066

BIODIVERSITY OF FULGOROMORPHA (HEMIPTERA) : HOW MANY SPECIES ARE THEY ?

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Managing intelligently biodiversity studies will not be the same with 10 or 100 millions of species. However, insect species number ranges from 8 to 100 millions (Groombridge, 1992). In such a frame, the question of specific richness appears as one of the first to answer.

Attempting to know the number of living species, scientists have developed different methods. Two main kinds of approaches have been tried : the number of species versus area and the number of species versus time (number of described species per year). It is clear that both approaches cannot provide a correct estimate because human constraints are strongly linked with the data available. However some land marks or tests may help to control the accuracy of the resulting estimates.

In order to illustrate this approach the Hemiptera Fulgoromorpha have been taken as a model.

Hemiptera Fulgoromorpha are an old lineage probably originating from Archaeoscytinoidea around the end of the early Permian ; modern fulgoromorpha groups (Cixiidae, Achilidae) do not appear until the Cretaceous (Scherbakov, 1988). Twenty families, mostly tropical, are currently recognized within the Fulgoromorpha. The whole of described species has increase from 7093 (given in Metcalf's catalogues published between 1932 and 1958), to 9200 (Woodward et al, 1970) and more than 10000 in the last edition of Insect of Australia (Carver et al., 1991). It amounts 10571 at the end of 1993.

Most of the trend curves of the number of species described each year by family show a sigmoidal form. From all the models tested by previous authors and from those available in mathematical literature, the logistic and the Gompertz models fit the best with the observed data. Nevertheless both do not provide the same range of number of species by family. More complex models using inflexion point estimation are thus proposed.

15-065

THE DISPERSAL OF FULGOROIDEA (HOMOPTERA) TO OCEANIC ISLANDS.

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Fulgoroidea are disproportionally common on oceanic islands compared to other Auchenorrhyncha. However, their occurrence by family does not reflect their relative species abundance or population abundance on continents. The presence of Delphacidae, with the greatest number of species on islands, is expected because they represent 97.8 % of the aerial plankton collected over oceans by Gressett et al., with Cixiidae 0.1% and Derbidae 2.1%.

Five of the most commonly found families other than delphacids, Cixiidae, Derbidae, Achilidae, and the Kinnaridae-Meenoplidae pair, may have a competitive advantage for rafting because nymphs are found under bark or in holes in logs or in soil. Five other families found on islands (Tropiduchidae, Issidae, Flatidae, Nogodinidae and Ricaniidae) do in some genera oviposit within plant tissue.

The family Fulgoridae has no representatives on small oceanic islands, nor do five other small families. Eurybrachidae and Dictyopharidae have one and two species respectively.

At least 2/3 of the species are endemic to their island. There is no correlation between the percent of polyphagous species in a family and its success in colonizing islands.

15-067

INSECT PEST CONTROL BY CAMBODIAN RICE FARMERS

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Over 1000 lowland rice farmers, 400 upland rice farmers, and 400 deep water rice farmers were interviewed throughout Cambodia to determine farmers' pest control practices. Over 100 farmers were interviewed per province. Data was analyzed by province as well as nationally. In the province of Kampong Cham, 12 to 13 times as many farmers grow lowland rice in the wet season as in the dry season. Less than 20% of these farmers use insecticides during the wet season, while over 80% use insecticides during the dry season. There are currently no laws regulating the import, sale, or use of pesticides in Cambodia. Most of the pesticides used in Cambodia are WHO Class I insecticides, which are usually banned or restricted in other countries. Farmers employ a combination of biological control, cultural control, home-made botanicals, and insecticides to manage rice pests. This paper will describe each of these methods in relation to sociodemographic factors, with particular emphasis on those pest control techniques unique to Cambodia. In addition, there will be a discussion of the sources of insecticides, the types of insecticides available, and the quality of insecticides in Cambodia.

15-068

SOYBEAN CULTIVATION. INSECT PEST MANAGEMENT. & BENEFIT /COST RATIOS IN JAPAN

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Soybean was cultivated in 68600ha with a production of 98500 metric ton(mt) and an avg of 1.81 mt/ha in 1995. About 271 invertebrates(245 insect, 12 nematode, 7 Stylopomatophoran, 5 mite, and 2 Isopod species), 9 birds, and 13 mammals are recorded as pests. In the south-western, insect pests are very serious: Lepidopterous defoliators (*Spodoptera litura*, *Plusia* sp. [Noctuidae], Dipterous podfeeder (*Asphodylia* sp. [Cecidomyiidae]), Lepidopterous podborers (*Etiella zinckenella* [Pyralidae], *Matsumuraesia* spp. [Tortricidae]), Heteropterous podsuckers [Pentatomidae and Alydidae], etc. occur. In Ibaraki, central part Japan, yield loss due to insect pests was assessed using 3 applications of Parmathion 40 (fenitrothion 30 + fenvalerate 10%) WP in treated plots in 1995. Under non-tillage for seeding, yields were 1.6 (relative value of 100) and 0.4t/ha(25) in treated & untreated plots, respectively. Under a common method or tillage for seeding, those were 2.0(100) and 1.2 t/ha (58), respectively. Thus, yield losses due to insect pests were estimated at least 75 & 42%, respectively. Benefit/cost ratios were 5.6 & 17.0 in non-tillage and tillage seeding fields, respectively.

15-070

THE SOYBEAN PESTS IN SOUTHWEST OF IRAN.

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In three years survey, the Soybean pests were studied in khuzestan province, southwest of Iran. Two different experimental fields were selected at Ahwaz Agricultural College field station. By different methods of sampling such as light trap and leaf sampling, the Soybean pests were collected and identified as follow:

- 1- *Bemisia tabaci* Gennad (Homop., Aleyrodidae).
- 2- *Tetranychus tukrestani* Ugarov and Nikolski (Acarina, Tetranychidae)
- 3- *Nezara viridula* L. (Hemip., pentatomidae).
- 4- *Aphis craccivora* Koch (Homop., Aphididae).
- 5- *Spodoptera exigua* Hubner (Lepidop., Noctuidae).
- 6- *Prodenia litura* Fabricious. (Lepidop., Noctuidae).
- 7- *Heliothis armigera* L. (Lepidop., Noctuidae).
- 8- *Thrips tabaci* Lin. (Thysanop., Thripidae).
- 9- *Agriotes* SP. (coleop., Elateridae).
- 10- *Anacridium aegyptium* L. (Orthop., Acrididae).
- 11- *Epilachn chrysomelina* F. (coleop., coccinellidae).

Moreover, the populations of *B. tabaci* and *T. turkestan* two important of Soybean pests were estimated.

15-069

FORECASTING OF POPULATION DENSITY AND OUTBREAKS IN *AELIA* AND *EURYGASTER* SPECIES (HEMIPTERA: HETEROPTERA: PENTATOMOIDEA).

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Fluctuations in population density from one year to another in the cereal pest species, *Aelia acuminata* L., *A. furcula* Fieb., *A. melanota* Fieb., *A. rostrata* Boh., *A. virgata* Klug., *Eurygaster integriceps* Put., and *E. maura* L. is related to the physiological condition of the individuals and ecological factors of biotic and abiotic nature. It is possible, from a study of the physiological condition of the internal organs of these species, to forecast the degree of their abundance during forthcoming invasions of wheat and barley fields.

Survival of individuals depends on the quantity and quality of food reserves (size of fat-body), from the time of migration to resting site (summer, autumn, winter) until they return to the fields during the spring of the following year.

A long term study of population density of *Eurygaster integriceps*, in two localities with different ecological conditions during 1960-1995 support the above hypothesis.

15-071

AN INVESTIGATION ON THE DISTRIBUTION OF *DOCIOSTAUROS* FIEB. SPECIES (ORTHOPTERA: ACRIDIDAE) IN WESTERN IRAN (ILAM)

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An investigation carried out on the distribution of *Doclostaurus* species in five districts of province revealed that at least five species and subspecies: *D. maroccanus* (Thunb.), *D. hauensteini hauensteini* (I. Bol.), *D. hauensteini elbursianus* uv., *D. anatolicus* (Kr.) and *D. jagoi* n. sp. with various populations are distributed in these regions, distributional map for these regions is prepared. According to these findings Mehran district in Ilam province is permanent breeding area of Moroccan locust, in outbreak area there will be more than one thousand nymphs in a square meters, it spends 8-9 months of the year as eggs in the soil, it takes 58-85 days from first instar to the end of oviposition Period.

As far as economic importance is concerned, *D. maroccanus* caused economic damage to many agricultural and forage crops and due to rather high population density applied methods of control is necessary to reduce its damage.

15-072

MONITORING THE FIRST FLIGHT ACTIVITIES OF CEREAL APHIDS USING SUCTION TRAPS AND COLOURED WATER TRAPS

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The primary colonization of cereals by aphids in autumn and spring is a critical event concerning the risk of BYDV infection. Prediction of population trends (i.e. settling, spread) is essential for risk evaluation. Basic data needed are time and intensity of the first immigration of the three species *Rhopalosiphum padi* (L.), *Sitobion avenae* (F.) and *Metopolophium dirhodum* (W.). However, visual controls and D-vac sampling are very time consuming since high numbers of replicates are needed due to the spatial distribution pattern during the immigration period. Therefore the suitability of stationary traps for monitoring was tested from 1993 to 1995.

During spring and summer 1995 yellow and green water traps were used to monitor the flight activity of cereal aphids in addition to three suction trap types (high-level: 12 m, low-level: 1.7 m, variable low-level: 0.5-1.5 m). In the 12-metre trap the first alatae of all three species were recorded at May 7th and 8th, in the lower suction traps 5-7 days later. The first cereal aphids were caught in coloured water traps a few days up to three weeks later. The first flight activity of *S. avenae* corresponded well in both types of colour traps in contrast to that of *M. dirhodum*. *S. avenae* was dominant within the crop which was reflected by the suction traps. *R. padi* however was much more dominant in the 12-m trap compared to the real abundance in the crop. Only in the green water traps the distribution of the species corresponded well with observations in the field. The data show that suction traps and coloured water traps can be used to measure time and intensity of immigration. Similar trends were previously observed for suction traps.

15-074

EFFECT OF DIFFERENTLY STRUCTURED FIELD MARGINS ON THE ABUNDANCE AND DISPERSAL OF CARABID BEETLES (COLEOPTERA: CARABIDAE)

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Long-term studies indicate drastic reductions in epigeic predatory arthropod densities due to intensification of farming practices, including fertilisation and pesticide use, and the reduction of diversely structured field boundaries. These results stress the importance of non-cultivated habitats as possible sources of restoration of field-dwelling beneficial arthropod fauna. The aim of this study was to estimate the importance of differently structured field margins on the abundance and migration of carabid beetles in and between agricultural and surrounding landscapes.

The study sites were situated on two winter wheat fields in Northern Germany. The fields were bordered by three different types of field margins (permanent succession, annual succession, sown wildflower mixture). All these boundary strips had a width of 3m and a length of at least 30m, with 4 replications on each study site. Estimates of absolute densities of carabid beetles were obtained by means of quadrat samples, taken both in the field margins and in the winter wheat. The activity density was recorded using pitfall traps. The dispersal of carabid beetles between crop and adjacent field margins was measured by directional pitfall trapping (gutter traps) and mark-recapture techniques.

The species composition and the abundance of carabid beetles differed between the crop free strips and the arable land. An exchange of carabids during the vegetation periods could only be recorded for some species. The mark-recapture estimates reveal the species-dependent permeability of field boundaries.

15-073

DOES STRAW MULCH PROTECT LUPIN CROPS FROM INCOMING APHIDS (HOMOPTERA: APHIDIDAE)?

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Studies of aphid landing show that in some species, fewer aphids land on plants where there is more ground cover. Trials were conducted to investigate the effect of ground cover (straw mulch) on aphid landing behaviour in lupin crops, with the intention of controlling aphid infestations and the spread of aphid-borne plant viruses.

Plots were sown with lupins and half the plots were covered with straw mulch (to simulate stubble retention), the other half had bare earth. Alighting aphids were collected from water traps positioned over the plots.

Significantly more aphids (up to three times as many) were caught in traps positioned in the bare earth plots than those with mulch, particularly early in the season when there were large gaps between plants. Aphids were less attracted to plots where lupin plants were interspaced with straw mulch. Differences in aphid landing rates between bare earth and stubble treatments decreased as the canopy cover increased.

Stubble will offer some protection to the crop from aphid attack, especially when aphids arrive early and the open canopy makes the crop more vulnerable. How does stubble alter aphid landing behaviour? Aphids respond to visual signals (wavelengths) emitted from the ground, in particular light reflectance. Some species of aphids were repelled by the higher proportion of light reflected by straw mulch than that reflected by bare earth.

15-075

DIVERSITY, ABUNDANCE AND PHENOLOGY OF PHYTOSEIID MITES (ACARI: PHYTOSEIIDAE) IN VINEYARDS AND ON THE SURROUNDING VEGETATION IN NORTH-EASTERN ITALY

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The mite fauna occurring in vineyards and on the surrounding vegetation was monitored in different areas of North-Eastern Italy. Phytoseiids were a major component of the mite communities on wild vegetation and in vineyards. A total of 13 different species were found on the wild vegetation. Leaf morphology appeared to be important in affecting phytoseiid colonization. Six phytoseiid species were found on grapevines throughout the sites. A correlation between the dominance of some predatory mites on wild vegetation and in vineyards was found in some cases and, in particular, the economically important *Amblyseius aberrans*, *A. andersoni* and *Typhlodromus pyri* were involved.

The Tydeidae were widely diffused on wild plants and in vineyards and the same species were frequently recorded on both. Phytophagous mites (Tetranychidae, Tenuipalpidae and Eriophyidae) were rarely abundant on wild vegetation and in vineyards. The fungivore Winterschmidtidae were common on wild plants but not in vineyards. In various localities and years the phytoseiid densities were significantly higher in mid season than in late season on the wild vegetation while they were more abundant in early season in vineyards. The mechanisms affecting the persistence and abundance of phytoseiids on wild plants may suggest strategies for successfully managing their populations in vineyards.

15-076

Impact of Rice Water Weevil, *Lissorhoptrus oryzophilus* Kuschel, (Coleoptera: Curculionidae) Larval Injury on Rice Plant Photosynthesis and Biomass Accumulation

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A 3-year field study was conducted in California to determine the effects of rice water weevil infestation on rice plant growth, development, photosynthesis, and yield. Differential larval densities were established by infesting from 0 to 0.6 rice water weevil adults per plant (retained with floating row covers). In 1993 and 1995, rice water weevil larval densities correlated closely with adult infestation levels. The average larval density increased from 1 to 17 per plant over the 0 to 0.6 infestation regimes. In 1994, treatments generally resulted in uninfested plots (a low background infestation of ~1 larva per plant) and in infested plots (averaging ~8.3 larvae per plant). The primary damage from this insect is from the larval injury to the root systems. Reductions in vegetative biomass accumulation were seen at infestation levels ≥ 0.4 adults per plant (= ~7 larvae per plant). The number of tillers per plant, and resulting leaf area per plant, was most severely reduced by rice water weevil larval injury. Rice photosynthetic rates were reduced by the root injury during the period of larval feeding; however, photosynthetic rates of injured plants elevated to that of uninjured plants at and following the time of pupation. This physiological response to root injury is similar to that recorded for other soil-borne insect pests. Grain yield was reduced by rice water weevil larval injury by up to 45% in 1993 and by 33% in 1995 by the highest infestation levels. A linear relationship best described the yield loss/larval density relationship with a 2.9% yield loss per larvae. In 1994, the maximum yield loss was 21%. It was not possible to form a significant relationship between larval density and yield because of the lack of a range of rice water weevil larval densities in 1994.

15-078

THE RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA* IN CHILE: DISTRIBUTION AND YIELD LOSSES

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The Russian wheat aphid (RWA) was first discovered in Chile in 1987. Because of heavy infestations and serious economic losses caused by the aphid in South Africa and USA, a survey together with yield losses assessments were conducted throughout the main small grain growing areas of Chile.

Presently, RWA occurs in most grain production areas of the country (Parallels 29°53' to 38°56'S), including localities with rainfall as nearly 1400 mm. However, the aphid has not adversely affected wheat production or quality in south-central Chile, except in extremely delayed plantings.

15-077

THE PEA LEAFMINER, *LIRIOMYZIA HUIDOBRENSIS* (DIPTERA: AGROMIZIDAE), AS A PEST OF LEAFY VEGETABLE CROPS IN THE SALINAS VALLEY OF CALIFORNIA, U.S.A.

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The pea leafminer has become the most serious insect pest of leafy vegetables in Salinas Valley of California. The reason for the change in pest status from a sporadic, easily controlled pest to one that is responsible for the destruction of many entire fields of spinach and an 300% increase in insecticide costs in lettuce has been examined in a number of field and laboratory investigations.

The role of insecticidal resistance, changing cultural practices, the parasitoid complex and host preference shifts in this pest species have all been examined. It has been shown that the leafminer populations in this area are resistant to many of the products used for their control. Resistance to permethrin applied to control adults was found to be so high as to lead to total field control failure. It was also shown that lettuce has become the most important host in the system for this pest. Whole plant samples from lettuce fields at harvest in August 1995 were found to have an average of 660 leafminer larvae per plant, despite 14 applications of insecticides applied to the field. The role of cultural practices and the parasitoids seem to be less important.

15-079

PREMATURE FRUIT DROP CAUSED BY THE ALMOND SEED WASP *EURYTOMA AMYGDALI* ENDERLEIN (HYMENOPTERA: EURYTOMIDAE) TO THREE ALMOND VARIETIES

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The main if not the only damage caused by the almond seed wasp, *Eurytoma amygdali* Enderlein (Hymenoptera: Eurytomidae), has been known to be the erosion of the seed by the larva. Infested fruit containing grown larvae become mummified and remain on the tree beyond harvest. To ascertain suspected additional damage to infested fruit in the form of premature drop, almonds on trees of three varieties were oviposited on different dates and for different periods of time by released adult wasps or by the natural orchard population, and fruit dropping were recorded weekly.

Infested fruit suffered heavy premature drop in Texas (Mission) and Ferragnes, but not in the Truoto variety.

The results suggest a change of the established strategy of control, to prevent substantial oviposition in varieties that suffer such premature fruit drop.

15-080

THE BIOLOGY OF *SEJANUS ALBOSIGNATUS* IN ORGANIC APPLE ORCHARD IN CANTERBURY, NEW ZEALAND: FRIEND OR FOE?

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The distribution of *Sejanus albosignatus* (Hemiptera: Miridae) in conventionally and organically managed orchards is described. The seasonal phenology was investigated over the summers of 1993/94, 1994/95 and 1995/96 in an organic orchard.

Characteristically, there are two generations per year. The first emerges from overwintering eggs in early spring, passes through five nymphal instars and reaches adulthood by early summer. The second generation, which is smaller than the first in size, emerges in late December - early January and adults appear by early February. Egg diapause appears to affect a proportion of the first generation eggs and all of the second.

The effect of herb understorey on the biology of *Sejanus* is discussed. The damage to fruit and the effect of apple variety on its incidence are described as is the mirid's predation on other apple pests.

15-081

NYMPH AND ADULT PERFORMANCE OF THE SOUTHERN GREEN STINK BUG (HETEROPTERA: PENTATOMIDAE) ON JAPANESE PRIVET COMPARED TO OTHER SELECTED FOOD PLANTS

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Laboratory studies with the southern green stink bug, *Nesara viridula* (L.) indicated that nymphs performed better when fed on immature fruits of soybean, *Glycine max* (L.) Merrill (Leguminosae), than on immature fruits of Japanese privet, *Ligustrum japonicum* Thunb. (Oleaceae). Female and male nymphs required 6.7 and 4.6 d more to complete development on Japanese privet than on soybean, with greater mortality (38.7%) on the former than on the later (20.0%) food plant. At emergence adult fresh body weight was ca. 34-40% greater on soybean than on Japanese privet. In contrast to nymphs, adults *N. viridula* performed better on Japanese privet than on soybean, with greater % of females ovipositing on the first (62.5) than on the second (45.0) food. Fecundity was 2-3X greater on Japanese privet [alone or supplemented with dried soybean seeds+shelled peanuts, *Arachis hypogaea* (L.)], than on soybean fruits or vegetative plants, or on water cress, *Nasturtium officinale* L. (Brassicaceae) leaves, these last two foods supplemented with dried soybean seeds+peanuts. Fresh body weight gain up to the 4th week of adult life was ca. 3X greater on Japanese privet than on soybean fruits. Total longevity of females and males was similar on Japanese privet or soybean fruits, but it was greater on Japanese privet than on water cress, both supplemented with soybean seeds+peanuts.

15-082

THE DRAMATIC SHIFT OF THE WESTERN CORN ROOTWORM, *DIABROTICA VIRGIFERA VIRGIFERA* LECONTE (COLEOPTERA: CHRYSOMELIDAE), TO MAIZE IN ROTATION WITH SOYBEANS IN INDIANA

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The primary means for managing the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in Indiana over the past 20 years has been through crop rotation. This has resulted in a soil insecticide usage-reduction of 340,500 kilograms of active ingredient per year.

In 1993, a few reports of rootworm larval feeding damage in maize fields following soybeans were received. Since rootworm eggs are laid in late summer prior to hatch the next spring, the adults had to have moved into soybean fields to lay their eggs. Each year since then, more farmers have observed this phenomenon. By 1995, more than one thousand maize fields were reported as having this problem.

It is difficult to say why this is happening. There is speculation that the rootworm beetle may be adapting to the maize/soybean rotational system. It could be that the maize residue on the soil surface is giving off volatiles that are attracting the beetles into the soybeans. Also, earlier maize planting and later larval hatch could be causing the maize and the insect to get out of sync with each other. Perhaps we have a biotype.

Part of the concern is that farmers will return to using soil insecticides as prophylactic treatments. If this happens, much of what we have gained in managing this insect with alternatives to insecticides will be lost. Studies are underway to help us determine why this is happening and what are the appropriate management strategies.

15-083

BEHAVIORAL ADAPTATION OF *HELICOVERPA ARMIGERA* TO THE COSMOPOLITAN WEED, *SONCHUS OLERACEUS* L. (ASTERACEAE)

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The common sowthistle, *Sonchus oleraceus*, is a native of Europe and North Africa, but has established in at least 56 other countries. Thus, the original distribution of *H. armigera* overlapped with that of *S. oleraceus*. *Sonchus oleraceus*, therefore, may well be a primary host plant of *H. armigera*. In other words, *H. armigera* may be behaviorally and physiologically adapted to exploited *S. oleraceus*.

Helicoverpa armigera females showed consistent preference for *S. oleraceus*, irrespective of their age and host in the field. Phytochemicals from *S. oleraceus* may play an important role in mediating the behavioral steps leading to selection of the host plant for oviposition, as the solvent extracts of *S. oleraceus* significantly attracted oviposition by *H. armigera* females. On the other hand, there was significant individual variation in oviposition preference and such variation was genetically based.

Empirical evidence from our study, along with field observations, supports the view that *S. oleraceus* is a primary host plant to which *H. armigera* became adapted at the time of speciation. Nevertheless, the existence of significant variation in oviposition preference presumably maintains sufficient flexibility for the observed levels of polyphagy in *H. armigera*.

15-084

LIFE TABLE AND INTRINSIC RATE OF INCREASE OF THE DIAMONDBACK MOTH *PLUTELLA XYLOSTELLA* (L.) LEPIDOPTERA: (YPONOMEUTIDAE) ON FIVE DIFFERENT COLE CROPS.

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Out of the major pests the diamondback moth has gained importance recently in Himachal Pradesh by damaging cole crops to a greater extent. In order to understand the population dynamics of the pest its life tables have been constructed on five different cole crops which are cabbage, cauliflower, khol-khol, Brussel's sprout and sprouting broccoli.

Life table studies revealed that the gross reproductive rate of the moth was 114.2, 148.8, 104.5, 153.7 and 157.9 female eggs per female on these crops respectively. In a true generation time of 25.2, 23.2, 25.7, 23.8 and 20.3 days, the moth multiplied 85.7, 116.0, 69.7, 131.1 and 118.9 times on the respective hosts. The true intrinsic rate of increase was maximum (0.236) on sprouting broccoli, thus it can be considered more suitable for rearing this pest in comparison to the other hosts. The finite rate of increase (λ) on these crops was found 1.19, 1.23, 1.18, 1.27 and 1.23 respectively.

15-086

BIOLOGY AND HOST PLANT RELATIONSHIPS OF *SCAPTOMYZA FLAVA* LEAF MINER

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The relationship between ovipositional preference of *Scaptomyza flava* Fallen (Diptera: Drosophilidae) and host plant suitability on seven host plant species (Chinese cabbage [*Brassica chinensis*], turnip [*Brassica rapa*], radish [*Raphanus sativus*], hedge mustard [*Sisymbrium officinale*], cauliflower [*Brassica oleracea* var. botrytis], lettuce [*Lactuca sativa*], wheat [*Triticum aestivum*] and prairie grass [*Bromus willdenowii*]) was evaluated. Ovipositional preference of *S. flava* was determined by measuring feeding punctures and egg density after adult female flies were given a simultaneous choice and non-choice of all host plants for feeding and oviposition. All studies were performed under greenghouse conditions. *S. flava* showed distinct hierarchical ordering in their ovipositional preference with turnip, Chinese cabbage and hedge mustard being preferred over all others. *S. flava* is an oligophagous insect with host plants restricted to Brassicaceae. In non-choice tests, females laid more eggs on Chinese cabbage and turnip other Brassicaceae. Egg production was also different between host plants. Females oviposited means of 255, 165 and 48 eggs during their lifespan when maintained on turnip, Chinese cabbage and cauliflower, respectively. There were also significant differences in total developmental times of the insect between three Brassicaceous host plants (cauliflower 41d. Chinese cabbage 33.7d and turnip 31d).

15-085

EFFECT OF LARVAL FOOD PLANT ON ADULT EGG LAYING PREFERENCE OF *SCAPTOMYZA FLAVA* (F.) (DIPTERA: DROSOPHILIDAE) LEAF MINER

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To evaluate the effect of larval food plant on adult egg laying preference *S. flava* was reared on 3 plant species, Chinese cabbage, cauliflower, and turnip.

In terms of total feeding punctures and eggs laid, flies reared on Chinese cabbage produced significantly more than those reared on turnip, which in turn produced more than those reared on cauliflower. This suggests that Chinese cabbage is the better quality host plant. Flies reared on turnip produced significantly more feeding punctures ($P \leq 0.01$) and deposited significantly more eggs on turnip than on Chinese cabbage or cauliflower. Flies reared on Chinese cabbage produced an equal number of feeding punctures on Chinese cabbage compared to turnip, but showed a distinct preference for Chinese cabbage for egg laying. However, flies reared on cauliflower showed no preference for cauliflower and made most feeding punctures and laid most of their eggs on Chinese cabbage. Thus, although there was significant preference for both egg laying and feeding on turnip in the case of insects raised on that plant and similar slight preference in the case of Chinese cabbage, this was not the case at all for cauliflower where rearing on that plant induced no preference.

15-087

FACTORS AFFECTING INTERSPECIFIC AND INTERCLONAL VARIABILITY IN APHID PERFORMANCE AND APHID-INDUCED PHYTOCHEMICAL RESPONSES IN WHEAT

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Changes were evaluated in the accumulation of hydroxamic acids (secondary metabolites related to aphid resistance of cereals) in both wild and cultivated wheat following infestation by two clones of the aphid *Sitobion avenae* (Fabr.) markedly differing in their ability to thrive in wheat lines. A statistical interaction between plant genotype and aphid clone feeding behavior was detected. Further experiments including the aphid *Rhopalosiphum padi* (L.) and using electropenetration graphs (EPG) enlightened the relationship between aphid feeding behavior and aphid-induced phytochemical responses. The importance of a threshold of infestation (number of aphids) required to induce phytochemical responses was determined.

15-088

INFLUENCE OF MAIZE VARIETY ON THE CLONAL SELECTION OF *RHOPALOSIPHUM PADI* (HOMOPTERA: APHIDIDAE)

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Maize varieties exhibit a large range of susceptibility to aphids. As aphids possess a large genetic polymorphism this could lead to a rapid selection of aphid clones able to thrive on resistant varieties. Our aim was to test this hypothesis by the use of RAPD-PCR to quantify the genetic distances between aphids collected on different varieties but on the same location. It appears that the genetic distances between aphid caught on the same plant is low (15 % of the DNA bands) but between plants of the same variety these distances could reach 38 % of the DNA bands recorded. At the variety level this distances do not increase very much although some clones seem to be more frequent on more resistant varieties.

15-090

NON-CHEMICAL REGULATION OF CEREAL LEAF BEETLE IN ORGANIC GRAIN FIELDS IN EASTERN AUSTRIA

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The Cereal Leaf Beetle CLB (*Oulema melanopus* L. Chrysomelidae) is an important pest of organic grain production in Eastern Austria.

The effectiveness of preventive cultivation measures (choice of cultivar, compost fertilization) and antagonists in reducing the CLB infestation levels have been investigated in an organic farm in Vienna.

Several cultivars of wheat were tested on CLB susceptibility. Different fertilization regimes (compost, inorganic fertilizers, combined fert.) were investigated in a plot trial towards their effects on CLB population dynamics and feeding damage. Egg-parasitization rates were measured in different vicinity of surrounding natural habitats.

From the obtained results, a non-chemical pest management strategy is derived.

15-089

EFFECT OF CULTIVATION PRACTICES ON THE INCIDENCE OF *HYPOLIXUS HAERENS* (BOHEMAN) (COLEOPTERA: CURCULIONIDAE) ON CULTIVATED AMARANTHS IN SOUTH AFRICA

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Hypolixus haerens (Boheman) seems to be the major stem-boring pest of cultivated amaranths in South Africa. The infestation levels of these weevils were determined at fortnightly intervals for 16 weeks on two similarly cultivated trials of which one received irrigation and the other one was rainfed. Within each trial four fertilizer treatments (viz. no fertilizer, cattle manure, wood ash and chemical fertilizer) were applied in order to simulate growing conditions varying from subsistence to commercial farming. Statistical analysis of the data revealed that significantly more ($p < 0.05$) adult weevils occurred in the rainfed than in the irrigated trial during the first six observations. During the last two observations no significant differences in insect numbers were recorded. No significant differences ($p < 0.05$) in weevil numbers were recorded between the various fertilizer treatments in both trials. The within-season distribution pattern of *H. haerens* was determined by plotting weevil numbers against time. Two definite peaks of occurrence were discernible viz. mid-December and mid-January. Weevil numbers declined sharply from the second week of February, coinciding with the decline in vegetative plant growth. The within-distribution patterns were similar for both trials.

15-091

EVIDENCE ON GEOGRAPHICAL DIFFERENCES IN MALE RESPONSES TO SYNTHETIC PHEROMONE BLENDS IN THE LIMABEAN POD BORER (*ETIELLA ZINCKENELLA* TREITSCHKE) (LEPIDOPTERA: PHYCITIDAE)

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The limabean pod borer (*E. zinckenella*) is an important pest of fabaceous crops in regions with hot and temperate climate of Europe, Africa, Asia, Australia and the Americas. Several components have previously been identified from the female-produced sex pheromone of populations in Central Europe and Northern Africa. In the present study, field trapping tests were conducted at several geographically distant sites of the area of distribution of the pest using (Z)- and (E)-11-tetradecenyl acetate (Z11-14Ac; E11-14Ac), (Z)-9-tetradecenyl acetate (Z9-14Ac) and some binary and ternary mixtures of these as baits. At sites in Europe very good catches were observed with maximal activity in baits containing a 100:3 mixture of Z11-14Ac and Z9-14Ac. Some activity was also recorded at the above combination in India, whereas at sites in Eastern Asia or in Australia no male catches were recorded to any of the baits. The present results suggest that there are geographical differences in pheromonal response of *E. zinckenella* populations in different areas. At present it seems that pheromone-baited traps developed for monitoring of the pest in Europe and Northern Africa cannot be used in Eastern Asia or Australia, where a reinvestigation of the local populations' pheromone composition is needed in order to develop a pheromone-based monitoring trap.

15-092

CONTROL EFFECT OF A SYNTHETIC SEX PHEROMONE AGAINST COMMON CUTWORM, *SPODOPTERA LITURA* FABRICIUS (LEPIDOPTERA: NOCTUIDAE) ON ROSE IN GREENHOUSE DURING THEIR OVERWINTERING PERIOD

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In rose culture in southern Japan, some kinds of mites, aphids and Common cutworm are dominant. Chemicals are frequently sprayed to control these pests especially the latter. We reported on the practical control effects of the mating communication disruption by using sex pheromones against Lepidopteran pests in greenhouses. The results showed that the effects in greenhouses were greater than those previously reported by others in open spaces, and that the population density was more intensely depressed during their overwintering period than in summer season. The present experiment was conducted to confirm these results in commercial rose greenhouses from October 1989 to January 1990. Five hundred pheromone dispensers were set in a treated house (1000 m², above 17°C) and none in a non-treated one. One month later, the damage ratio of plants in the treated house quickly decreased from 22% to 12%, in contrast to its increase in the non-treated one. The damage ratio in the former kept lowering until the winter, becoming finally zero. In the non-treated house, however, the damage ratio did not lowered to zero. It was concluded that Common cutworm could easily be eradicated by using the sex pheromone in low population density.

15-094

HOST PLANT VOLATILES SYNERGISTICALLY ENHANCE THE CAPTURE OF MALES IN COMMERCIAL SEX PHEROMONE TRAPS FOR VARIOUS MOTH SPECIES

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Since female moths call and release their sex pheromones while perched on their host plants, we isolated and identified host plant volatiles (HPVs), and tested various HPVs for their ability to attract moths and/or modulate the attraction of males to synthetic commercial sex pheromones in both field and orchard crops. To test their inherent attractancy, individual HPVs were placed alone in traps and were found to be nonattractive to both male and female moths. To test the effects of HPVs on sex pheromone attraction, pairs of pheromone-baited traps were placed in close competitive positions, with one of the pheromone traps augmented with a HPV, the other without. When co-emitted with sex pheromone from baited traps, various HPVs were found to synergistically enhance the male attraction and capture efficacy of the pheromones by between 20% to over 300%. The HPVs acted as sex pheromone-synergists, increasing the capture of exclusively male moths and not causing attraction/capture of female moths. Various HPVs were found to synergistically enhance the attraction of commercial pheromones of a wide range of moth species, including Noctuidae (*Helicoverpa zea*, *Heliothis virescens*, *H. phloxiphaga*, *Trichoplusia ni*, *Spodoptera exigua*, *Pseudaletia unipuncta*, *Agrotis ipsilon*, *Peridroma saucia*), Gelechiidae (*Anarsia lineatella*, *Pectinophora gossypiella*), Tortricidae (*Grapholita molesta*, *Platynota stultana*), Olethreutidae (*Cydia pomonella*), Pterophoridae (*Platyptilia carduactyla*), and Plutellidae (*Plutella xylostella*).

The HPVs identified as pheromone-synergists were of various chemical structure, ranging from hydrocarbon monoterpenes and sesquiterpenes to aliphatic alcohols, aldehydes, acetates, and acids. A number of different compounds were identified as pheromone-synergists for various species, e.g. 16 for *H. zea*, 8 for *A. lineatella*, 4 for *T. ni*, and 3 for *G. molesta*.

Night-vision assisted observations in the field suggest that the HPV-enhanced pheromone trap capture of males was due to increased attractancy of pheromone traps and not increased alightment/ensnarement in traps. And for *H. zea*, HPV pheromone-synergists significantly lowered the amount (approx. 50 fold) of synthetic pheromone required to elicit a threshold attractive response in the field.

15-093

SEX PHEROMONE OF THE TOMATO PEST *SCROBIPALPULOIDES ABSOLUTA* (LEPIDOPTERA: GELECHIIDAE)

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The major sex pheromone component of the South American Tomato Moth, *Scrobipalpuloides absoluta* (Meyrick), was shown recently to be (3E,8Z,11Z)-3,8,11-tetradecatrien-1-yl acetate. Wind tunnel bioassays indicated that a dispenser loaded with 1000 ng of the synthetic ester was found to be highly attractive to conspecific males. In the field, a initial series of experiments was carried out to evaluate the efficacy of five trap designs. In another series, the best trap, baited with 100 µg of the pheromone, caught on average 1200 males per trap per night, while those baited with one-day-old virgin female caught only 201 males. The male response to this pheromone is restricted to the same early-morning time window during which females exhibit calling behavior. The high biological activity of the synthetic pheromone suggests that it will prove useful in monitoring the pest population, and that it may also be useful in mating disruption.

15-095

FIELD TESTS WITH SYNTHETIC PHEROMONE-BAITED (CLICK BEETLE) TRAPS (COLEOPTERA: ELATERIDAE) IN TWO EUROPEAN COUNTRIES.

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Pheromone-baited traps were tested in Italy and Hungary for catching *Agriotes sputator* L. (geranyl butanoate), *Agriotes ustulatus* Schaller (E,E-farnesyl acetate) and *A. litigiosus* Rossi (geranyl isovalerate). Large numbers of *A. sputator* (Hungary) and *A. litigiosus* (Italy) were captured in the respective traps, while high numbers of *A. ustulatus* were caught in both countries, confirming earlier trapping results in Russia. Traps baited with females caught less *A. ustulatus* than those with synthetic bait traps in a preliminary comparison of female-baited vs. synthetic baited traps. Additional tests in Hungary showed that large numbers of *A. rufipalpis* Brullé can be caught in traps baited with geranyl hexanoate, while the same compound captured specimens of *A. sordidus* Illiger in Italy. These are the first indications on the pheromone composition of these species. Preliminary studies on optimal trap design and dosage were also performed on the above species. Recently acquired knowledge regarding *Agriotes* biology and rearing methods, proved to be essential to carry out effective studies on the synthetic pheromones. The latter can be considered suitable for monitoring the swarming period of different *Agriotes* species and giving usable information for Integrated Pest Management.

15-096

A FEMALE-PRODUCED SEX PHEROMONE FOR THE BUG *PHYTOCORIS RELATIVUS* FALLÉN (HEMIPTERA: MIRIDAE).

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The bug *Phytocoris relativus* Fallén has been associated with nut damage in pistachio orchards in California. Bugs can move into a crop quickly, and infestations can be difficult to detect by currently available sampling methods (e.g., beating tray samples) until significant damage has occurred. Consequently, our goal was to identify attractants for use in monitoring *P. relativus* populations.

Preliminary investigations determined that virgin female bugs were attractive to males. Comparison of volatiles collected from virgin females and males revealed that females produced five sex-specific compounds. A series of field bioassays was used to determine that only two female-produced compounds were required for attraction of males. Furthermore, the attraction was highly specific; only mature conspecific male bugs were attracted by the pheromone.

15-098

INSECTICIDAL TOXICITY AND REPELLENCY PROPERTIES OF NEEM EXTRACT (AZADIRACHTIN) ON QUEENSLAND FRUIT FLY *BACTROCERA TRYONI* (FROGGATT) DIPTERA : TEPHRITIDAE

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A methanolic neem extract (7% Azadirachtin) was evaluated for its efficacy as an insecticide to control eggs, larval instars and pupae of Queensland fruit fly *Bactrocera tryoni* (Froggatt). One hundred specimens of each life stage (egg, first instar, second instar, third instar and pupa) were dipped for 60 seconds in 80 ppm, 100 ppm, 120 ppm and 140 ppm, respectively. A 60 second dip in 400 ppm dimethoate, a standard fruit fly treatment, was used as a basis for comparison. Eggs and larval stages were retained on carrot media at 26°C until adults emerged. Pupae were held at 26°C on moist filter paper until exclusion. The number of emerged adults was recorded to evaluate survival.

Repellency of methanolic neem extract at concentrations of 80 ppm, 100 ppm, 120 ppm and 140 ppm was evaluated against adults of Queensland fruit fly on persimmons. Fruit was dipped in extract for 90 seconds and subsequently exposed to caged mature flies for 2 hours. Untreated persimmons were exposed concurrently. The number of egg batches per fruit was evaluated as an indication of repellency efficacy. Statistical evaluation of the data showed no significant differences between dipping at different concentrations or between untreated fruit. Differences between treated and untreated fruit were significant at the P = 0.01 level. The mean of egg batches recorded in untreated fruit ranged from 17.8 to 26.8. Mean egg batches for fruit dipped in 80, 100, 120 and 140 ppm were 3.2, 3.4, 2.0 and 2.8 respectively.

15-097

BOTANICAL INSECTICIDES: STUDIES ON AN OIL EXTRACTED FROM IDIOBLAST CELLS OF THE AVOCADO FRUIT

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Natural plant compounds have proven to be effective in insect control. A subset of these compounds, antifeedants, offers a potential approach for pest management by rendering plants unattractive or unacceptable to pest insects. *Spodoptera exigua* (Hübner), a generalist feeder and a major pest in many vegetables in California, has a long history as a key herbivore on lettuce, celery, and tomato. In an attempt to find alternatives to conventional chemicals for *S. exigua* control, the insecticidal activity of an oil extracted from idioblast cells of the avocado fruit was evaluated. In diet incorporation studies, the oil was toxic to both early (LC₅₀ = 0.16% by volume) and late instars (LC₅₀ = 0.51%). Larval weights of third instars were significantly reduced after feeding for 5d on diet containing 0.30% oil (28.95 mg/larva) as compared to a control diet (231.91 mg/larva). The oil also acted as a feeding deterrent. Choice tests initiated with neonates showed a significantly greater proportion of larvae on control diet rather than oil-containing diet for concentrations of 0.10% or greater. Similar results were obtained for choice tests initiated with third instars for oil concentrations of 0.20% or greater. In these tests the larvae consumed significantly more diet than treated diet. Experiments to determine the active compounds in the oil are being performed.

15-099

EVALUATION OF SOME EUPHORBIA SPECIES EXTRACTS AS A TOXIC BAIT AGAINST THE BLACK CUTWORM *AGROTIS IPSILON* (HUFN.).

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Acetone and ether extracts of *E. paralias* and *E. papulus* gave 100 % larval mortality to the 2nd instar larvae of *A. ipsilon* after 10 days from feeding of the treated bait diet containing 5 % extract concentration. Also ether extract of *E. retusa* and *E. geniculata* causes 100 % mortality after 15 days. Alcoholic extracts of *E. geniculata*, *E. papulus*, *E. helioscopia*, *E. retusa* and *E. paralias* gave retradation in the larval development and causing percentage of total mortality through larval and pupal stages ranged from 30-60 %. The most effective extract of the five tested Euphorbia species were acetone followed by ether and hexane. The isolated materials from *E. species* (Fatty alcohol, B-Amyrin and B-sterol) were more toxic at 0.1 % conc. in the bait died against *A. ipsilon* where 100 % mortality for the larvae were observed after five days from feeding on the treated bait diet.

15-100

EFFECTS OF IMIDAZOLE FUNGICIDES AND PLANT GROWTH REGULATORS ON FEEDING AND CYTOCHROME P450 IN A HERBIVOROUS INSECT, *SPODOPTERA ERIDANIA* (CRAMER) (LEPIDOPTERA: NOCTUIDAE)

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Fungicides and herbicides that interfere with cytochrome P450 in fungi and plants also interfere with cytochrome P450 in insects. Several of these compounds, uniconazole, diniconazole, propiconazole, triadimefon, and pachlobutrazole inhibit cytochrome P450 activities *in vitro*. Some of the compounds induce activities *in vivo*. Some of the compounds are feeding deterrents for *S. eridania*. These interactions can have significant practical effects on insects in fields where several pesticides are applied.

15-102

THE EFFECTS OF FEEDING PROTEASE AND α -AMYLASE INHIBITORS, SINGLY AND IN COMBINATIONS TO THREE SPECIES OF LEAFROLLERS (LEPIDOPTERA: TORTRICIDAE)N.P. Markwick¹, W.A. Laing¹, J.T. Christeller², J.Z. Maxwell³ & M.R. Newton¹

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The larvae of three leafroller species - *Epiphyas postvittana* (Walker), *Planotortrix octo* (Dugdale) and *Ctenopseustis obliquana* (Walker) - were chronically fed six digestive enzyme inhibitors in artificial diets over a two-week period from hatching, and their growth rate and digestive enzyme activities were measured. The inhibitors were fed singly and in combinations set to target most of the digestive enzyme groups present in these leafrollers. Of the four protease inhibitors (PIs), three were extracted from potatoes (POT1, 2, and 4) and the fourth was bovine pancreatic trypsin inhibitor (BPTI). The two α -amylase inhibitors (α Als) were extracted from wheat, the dimer (WD) and tetramer (WT). Previous trials had shown these inhibitors to be the most effective from a range of 21 PIs and 6 α Als.

POT1 significantly affected the growth rate of all three leafrollers and BPTI was effective against *E. postvittana* and *P. octo*. Most combinations of inhibitors did not reduce the growth rate further than the single most effective constituent, i.e. inhibitors presented singly in the diet were generally more effective in reducing the growth rate. One exception to this was a combination of POT1, BPTI and WT against *P. octo*.

All digestive enzyme activity was affected by the presence of the inhibitors in the diets. α Als increased α -amylase activity between 1.5 and 10 times. BPTI, either singly or in combination, dramatically reduced trypsin activity (10 times) and raised elastase activity (about 1.5 to 3 times) in all species. Since larval growth rate was so little affected whilst all enzyme activities were greatly altered, the question of compensation of one digestive enzyme for another, is discussed.

15-101

PHYTOCHEMICALS WITH ANTIFEEDANT ACTIVITY AGAINST CEREAL APHIDS

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The bird cherry-oat aphid (*Rhopalosiphum padi* L.) and the grain aphid (*Sitobion avenae* F.) damage their cereal host plants directly, during feeding, and by acting as vectors of barley yellow dwarf virus. Both feeding and virus transmission occur when the aphid stylets are in contact with phloem sieve elements, but application of antifeedants to leaf surfaces can deter the insects at an earlier stage in host plant selection. Phytochemicals from a variety of sources were prepared at 0.1% in ethanolic solution and painted onto excised oat leaves. Groups of aphids were offered treated leaves in choice tests with control, ethanol-treated leaves. Several compounds caused antifeedant effects on both aphid species, with significantly more individuals recorded on control leaves than treated leaves after experimental access periods of 15min-24h. Treatments which were active in choice tests (a drimane sesquiterpenoid, (\pm)-polygodial, an indolizidine alkaloid, castanospermine, and α and β hop acids) were also presented in no-choice tests, where insects had no access to control-treated leaf material. Some compounds were no longer effective in no-choice conditions, but treatment of leaves with polygodial and the hop β acid colupulone effectively reduced the number of aphids settling.

15-103

EFFECTS OF AN AZADIRACHTIN BASED COMPOUND ON THE FECUNDITY OF MEDITERRANEAN FRUIT FLY *CERATITIS CAPITATA* (WIEDEMANN) (DIPTERA: TEPHRITIDAE): STRUCTURAL AND ULTRASTRUCTURAL ANALYSISM. Cristofaro, V. Di Ilio, D. Marchini¹, P. Nobili, R. Dallai¹, U. Cirio

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The aim of this work was to analyze the effects of a neem extract on the fecundity of adults of *Ceratitis capitata* under laboratory condition.

Several compounds with biological activity were isolated from the neem tree (*Azadirachta indica* A. Juss, Meliaceae). The most known of them is a tetranortriterpenoid named azadirachtin, producing its action in different ways, such as antifeedant, interfering with the oogenesis, with the larval development and with the metamorphosis.

A series of no-choice tests were carried out in order to observe the induced influence on the development and on the functionality of the female reproductive system. Adults of med fly were fed with an aqueous solution of neem extract mixed with the rearing diet.

The oviposition rate of treated females was extremely low, versus a longevity rate statistically not different from the control.

Females treated since their emergence or after sexual maturity showed atrophic or abnormal ovaries with disorganized ovarioles. Nurse and follicular cells appears to be modified in number and polarity. Vitellogenesis and choriogenesis were altered with oocytes showing an irregular shape due to the paucity of yolk material.

15-104

IMPACT OF *BACILLUS THURINGIENSIS* INSECTICIDES ON THE POPULATION DYNAMICS OF COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA*, AND ITS PRINCIPAL PREDATOR *COLEOMEGLA MACULATA* (COLEOPTERA: CHRYSOMELIDAE - COLEOPTERA: COCCINELLIDAE)

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In field studies in 1992 and 1993, plots of comparable densities of overwintered Colorado potato beetle (CPB) adults were treated with *Bacillus thuringiensis* (Bt) insecticides. As a control, a number of plots were left untreated. Oviposition and larval population development of CPB as well as predation on CPB egg masses and densities of *C. maculata*, the principal predator of CPB in North Carolina, were monitored throughout the oviposition period of 1. generation CPB adults.

During both years, CPB egg mass recruitment declined earlier in Bt-treated plots than in untreated control plots. Further, significantly fewer large CPB larvae developed in Bt-treated plots than in the untreated control. No impact of the Bt-treatments or the earlier decline in prey densities (CPB egg masses) in Bt-treated plots on *C. maculata* abundance was observed during first generation CPB population dynamics. Consequently, predation of CPB egg masses was not affected by the Bt-treatments. The utilization of Bt-insecticides was compatible with naturally-occurring biological control in North Carolina potato crop systems.

15-106

RAPD-PCR ANALYSIS OF GENETIC VARIATION IN SWEETPOTATO WHITEFLY (*BEMISIA TABACI* GENNADIUS, HOMOPTERA: ALEYRODIDAE) POPULATIONS

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Individual whiteflies from Mexico, Guatemala, Venezuela, Bermuda, Puerto Rico, Brazil, Ecuador, Dominican Republic, India, Nicaragua, Chili, Spain, Germany, Israel, Zimbabwe, Pakistan, and several areas in the United States have been analyzed for variation in RAPD-PCR DNA banding patterns. These collections represent 50 different geographic locations or hosts. RAPD patterns were examined for each of three different 10-mer primers. Each RAPD-PCR run contained three to five individuals from samples of whiteflies collected in Arizona from 1981 - 1986 to represent the "A-type" RAPD patterns, plus three to five individuals from collections made in 1994 and 1995 in a three-acre cotton field adjacent to the Western Cotton Research Laboratory to represent the "B-type" RAPD patterns along with one to five individuals of the various collections mentioned above. Samples of whiteflies from Puerto Rico, Mexico, and India contained individuals which yielded the "A-type" patterns, but this occurred only rarely. Genetic analysis of the patterns showed that most of the collections did not fall clearly within the "A" or "B" types, but rather fell in a continuous distribution of genotypes between those two extremes. Viewed as world-wide organism, *B. tabaci* may be a variable collection of heterogeneous populations rather than a homogeneous collection of genotypes.

15-105

DISCOVERY OF MALE ACOUSTIC SIGNALS IN WHITEFLY GENERA, *BEMISIA*, *TRIALEURODES* AND *PEALIUS* (HOMOPTERA, ALEYRODIDAE)

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Courtship-like signals produced by male whiteflies during mating behavior were recognized for the first time. Synchronous audio-video recordings ascertained that sexually matured males of *Bemisia tabaci*, *B. argentifolii*, *Trialeurodes vaporariorum* and *Pealius rubi* produced substrate-borne vibrations by rapidly oscillating their abdomens in a stereotyped rhythm accompanied with a short moving action around the female. Signals of *B. tabaci* and *argentifolii* were a single burst with a high frequency modulation. *B. tabaci* is characterized by a longer burst of 443.4 ± 21.0 msec duration, modulated only on posterior 1/3, with 6 harmonics of 252 ± 10.8 Hz fund. frequency. *B. argentifolii* is characterized by a shorter burst of 258.0 ± 16.4 msec duration, modulated on posterior half, with 3 harmonics of 331.6 ± 13.9 Hz fund. frequency. *T. vaporariorum* is consisted of a sequence of discrete bouts of chirps with 10~20 pulses, varying in number in the courtship progress, with 255.8 ± 11.7 Hz fund. frequency and 1081.8 ± 82.2 msec duration. *Pealius rubi* is characterized by a single burst of constricted trimodal waves, of 120~124 msec duration and about 305 Hz peak frequency. Prolonged courtship behavior might be due to the necessity of sexual stimulation for female by repeated emission of the male acoustic signals. These acoustic data could be used to separate the species precisely, and were assumed to be a species-specific cue towards female in the premating behavior.

15-107

INTRA-AND INTER-CROP DISPERSAL OF THE COTTON WHITEFLY *Bemisia tabaci* Gennadius IN POLYCROP SYSTEMS IN TAMIL NADU, INDIA.

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The cotton whitefly, *Bemisia tabaci* is a serious pest in this part of the country. The polyphagous nature, high fecundity, quick dispersal and undefying behaviour with chemical pesticides has led to total loss of crops. Behavioural patterns of the adults towards sticky traps, barrier crops and *in situ* observations were studied.

The dispersal pattern depended on the availability of host crop in agroecosystems. Intra crop movement was more within a height of 30 cm from soil level. Ladies finger (*Abelmoschus esculentus* Linn.) Moench and egg plant (*Solanum melongena* Linn.) were favourable hosts during April-May. With the commencement of the monsoon during June-July the pest established on sesame (*Sesamum indicum* Linn.), sunflower (*Helianthus annuus* Linn.) and groundnut (*Arachis hypogaea* Linn.). From August it dispersed to cotton wherein outbreaks occurred. It also established on groundnut, sesame, sunflower and soyabean (*Glycine max* Linn.) during November to January. During off-seasons weed hosts viz., *Boerhaavia diffusa*, *Parthenium hysterophorus* and *Trianthema portulacastrum* sustained the pest.

It is observed that i) yellow sticky traps could be effectively used for monitoring (ii) there are seven broods in a year and (iii) maize (*Zea mays* Linn.), bajra (*Pennisetum typhoides* (Burm.) Stapf. & Hubb.) and sorghum (*Sorghum bicolor* Pers.) could be used as effective barrier crops to check the immigration and spread of the pest.

15-108

KINETICS OF TOMATO YELLOW LEAF CURL GEMINIVIRUS (TYLCV) DNA IN THE WHITEFLY VECTOR *BEMISIA TABACI* (GENNADIUS) (HOMOPTERA: ALEYRODIDAE)D. Bosco, P. Caciagli¹

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The kinetics of TYLCV DNA were studied in the vector *Bemisia tabaci* (Gennadius) by means of quantitative chemiluminescent dot-blot assay, using digoxigenin-labeled specific DNA probes. DNA amount was calculated by calibration of each membrane with a reference standard of viral DNA. Large batches of female whiteflies were allowed to feed for 12, 24 or 48 h on TYLCV-infected tomato plants, then transferred to cucumber plants, immune to the virus. Insects were sampled at different times during and after acquisition. The analysis was done either on whole insects or on head+thorax (including salivary glands) and abdomen separately.

The proportion of insects having a detectable amount of viral DNA (positives) was more than twice the proportion of insects, taken from the same population, which transmitted TYLCV in infectivity tests (infectives). The maximum amount of viral DNA, averaging from 0.5 to 1.6 ng per positive insect, was always attained at the end of the acquisition. The mean amount then decreased by about 2% per day, remaining clearly detectable up to 20 days from the end of acquisition, whereas insects ceased to be infective 13 days earlier. Analysis of dissected insects showed that only some of the whiteflies with positive abdomens had positive head+thorax. The proportion of the head-thorax positive insects was similar to the proportion of infective insects. In both parts of the body, the viral DNA remained detectable up to 18 days after the end of acquisition, showing that the virus remains in different insect tissues much longer than infectivity would indicate.

15-110

SEASONAL AND ANNUAL DYNAMICS OF *BEMISIA TABACI* (GENN.) POPULATION RESPONSES TO INSECTICIDES IN CALIFORNIA (HOMOPTERA: ALEYRODIDAE)S. J. Castle, N. Prabhaker¹, T. J. Henneberry², N. C. Toscano¹

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The B biotype of *Bemisia tabaci* was introduced into California sometime in the late 1980s, with infestations initially limited to floricultural operations in greenhouses. The first agricultural infestations were detected in the Imperial Valley in 1990, leading to the destructive outbreak of 1991 that caused \$129 million direct crop losses, principally to muskmelons, broccoli, cauliflower, cabbage, lettuce, cotton and alfalfa. The breadth of crops damaged in 1991 and in subsequent years in the Imperial Valley reflect the broadly polyphagous nature of *B. tabaci* and the potential for damage in far-ranging agricultural regions with warm-temperate or subtropical climates.

The succession of host crops of *B. tabaci* in the Imperial Valley throughout the annual cycle has made possible year-round monitoring of their responses to insecticides. Beginning in 1993, populations have been sampled seasonally from three crops: spring muskmelons, summer cotton and fall-winter cruciferous crops. Bioassays of field-collected adult whiteflies have been performed for various insecticides as either single or mixed treatments using the yellow-sticky card technique. This bioassay method uses 7.6x12.7 cm yellow cards lightly coated with Tanglefoot[®], then sprayed with a concentration of insecticide representing one of 5-6 doses for each treatment. Collections of adult *B. tabaci* are facilitated by their attraction to yellow, alighting on the sticky cards after being flushed from crop foliage. Mortality is scored 24 h later under a microscope, and LC₅₀s are generated by probit analyses and interpreted as the response of the sample population to a given insecticide treatment.

No progression to higher resistance levels was observed over three consecutive years (1993-95) of monitoring adult *B. tabaci* populations in the Imperial Valley. Regressions of LC₅₀s against time for each year's bioassay data indicated a lower mean response in 1995 compared to 1993 for bifenthrin and endosulfan in spite of continued use of both insecticides. Negative slopes significantly different from zero ($P < 0.05$) for the cotton crop LC₅₀s indicated higher susceptibilities at the end of the cotton season, whereas the opposite was true for the melon crop bioassay data. Differences in LC₅₀s between crop seasons suggested the potential role of host plant effects on adult whitefly susceptibilities to certain insecticides. Interseasonal dynamics of LC₅₀s suggest the involvement of ecological factors in addition to genetic factors in responses of *B. tabaci* populations to insecticides.

15-109

GENES ON BOTH DNA A AND B CONTRIBUTE TO ACQUISITION AND TRANSMISSION OF GEMINIVIRUSES BY THE WHITEFLY *BEMISIA TABACI* (GENNADIUS) (HEMIPTERA: HOMOPTERA: ALEYRODIDAE).

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African cassava mosaic virus (ACMV) and other bipartite geminiviruses have been used to investigate the molecular basis of transmission by the whitefly vector, *Bemisia tabaci*. Isolates and clones of insect transmissible and non-transmissible viruses have been compared biologically and at the molecular level. The biotype of whitefly and the feeding behaviour play a significant role. Viruses, which are potentially transmissible by the vector, can be acquired by non-vector species of whitefly and by other taxa. The coat protein of the virus determines if it will be transmitted. This is demonstrated by chimeric and pseudorecombinant DNA A components between different clones. Similar studies using chimeric or pseudorecombinant DNA B components showed that a movement protein plays a significant role in the acquisition of the virus. DNA B also affects symptom phenotype. ACMV symptoms differed with the genotype of the virus and they were reflected in the transmission results. In several cases, although an insect transmissible virus might cause severe symptoms, insects were subsequently unable to transmit. The environmental conditions, such as light and temperature, also affect the transmission process.

15-111

INSECTICIDE RESISTANCE MANAGEMENT OF WHITEFLIES IN IMPERIAL VALLEY, CA, USING INSECTICIDE ROTATIONS AND MIXTURES (HOMOPTERA: ALEYRODIDAE)

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In the USA, California and Arizona have been affected the most by intense and consistent infestations of *Bemisia tabaci*. Because of the temporal sequence and overlapping of crops, successive generations of whiteflies are exposed to insecticides on a continuous basis. Heavy reliance upon insecticides for reducing infestations in cotton and other crops heightens concern about the development of insecticide resistance in whitefly populations. To evaluate potential antiresistance strategies for whitefly populations, two principle approaches, insecticide rotations and insecticide mixtures, have been studied in both greenhouse and field settings. High levels of resistance (>500-fold) to bifenthrin were recorded for whiteflies in greenhouse colonies subjected to continuous bifenthrin exposure. Resistance increased to moderate levels (>30-fold) to endosulfan and chlorpyrifos under continuous selection pressure. However, only low levels of resistance (5- to 10-fold) were observed in colonies exposed to similar insecticide pressure, but in a rotational scheme using the same three insecticides. Similar contrasts in resistance levels were observed favoring mixtures of two insecticides over single compounds.

Field trials were conducted in 1994 at two sites in the Imperial Valley, CA, and in 1995 at sites in Imperial Valley and Yuma, AZ, to evaluate insecticide rotations and mixtures as resistance management strategies for whiteflies in an agricultural setting. Insecticide treatment regimens included continuous treatment plots with single insecticides using bifenthrin, endosulfan, chlorpyrifos and amitraz, rotation plots with the same four insecticides, a mixture treatment with bifenthrin and endosulfan, and untreated control plots. Ten consecutive weeks of bioassay results with the yellow sticky card technique failed to yield discernible differences in the insecticide treatment regimens (continuous, rotation, or untreated). However, there appeared to be a general trend of decreasing LC₅₀'s through time in most of the treatment plots. Whiteflies from Imperial Valley exhibited lower LC₅₀'s in 1995 to all the insecticides indicating they were more susceptible than in the previous year. Populations from Yuma were more susceptible than the Imperial Valley populations.

15-112

DENSITIES OF OVERWINTERING CARABIDS AND STAPHYLINIDS (COLEOPTERA: CARABIDAE AND STAPHYLINIDAE) IN CEREAL AND GRASS FIELDS AND THEIR BOUNDARIES.

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Abundances of carabids and staphylinids were measured during four winters in four fields with grass or autumn ploughed spring cereals, and in their boundaries. Most species were significantly more abundant in the boundaries than in the fields, indicating the high importance of boundaries in the agricultural landscape for these predator groups. Only the carabid *Clivna fossor* and staphylinids belonging to the genus *Lathrobium* were evenly distributed throughout the entire area.

As none of the species with higher populations in the grass fields and their boundaries were among the important predators of insect pests in agricultural fields, it was concluded that the grass fields were of limited importance as a reservoir for the predatory beetles.

15-114

STYLET ACTIVITIES ASSOCIATED WITH ACQUISITION AND INOCULATION OF BARLEY YELLOW DWARF VIRUS BY APHIDS.

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Phloem-restricted barley yellow dwarf viruses (BYDV) are acquired by aphid vectors during ingestion of phloem sap, and inoculated during injection of saliva into plants. We used the electrical penetration graph (EPG) technique to investigate the aphid stylet activities which are associated with acquisition and inoculation of BYDV-MAV by the grain aphid, *Sitobion avenae* (F.). Periods of intracellular stylet tip location are registered as potential drops during EPG recording; these may be short (all cell types) or long (phloem sieve elements only). Two waveforms occur during extended phloem sieve element penetration; firstly E1, which may be followed by E2 (E1+E2). All aphids which subsequently transmitted virus to oat test plants showed E1+E2 during recorded penetration of a BYDV-infected plant. Separate analysis of E1 and E2 durations suggested that E2 is associated with virus acquisition, as insects which transmitted to test plants showed longer total E2 occurrence on the infected plant than non-transmitting aphids. Most aphids which transmitted to a test plant during recorded inoculation access showed either E1 alone or E1+E2, and inoculations tended to coincide with longer periods of E1. However, some insects which transmitted virus showed E1 for <5min, and 10% of inoculations were effected by aphids that showed no E1/E2, indicating that transient phloem punctures occur which are indistinguishable from penetration of other cell types using EPG recording. The results suggest that BYDV-MAV acquisition by aphids requires sustained periods of phloem contact, but inoculation of the virus can occur during very brief punctures of phloem tissue.

15-113

INVESTIGATIONS OF EFFECTS OF CHANGING FARMING SYSTEM AND LANDSCAPE STRUCTURE ON THE GROUND BEETLES (COLEOPTERA, CARABIDAE) OF A LOESS SITE IN THE CENTRAL GERMANY DRY REGION

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A long-term research project is run to investigate the effects first of the transition from conventional to ecological farming and second of structuring the landscape by means of field baulks and hedges on the entomo-fauna. The following working hypotheses are tested:

- 1.: The insects of an agroecosystem are decisively influenced by the intensity of land management as well as by the landscape structure.
- 2.: The ecological farming and the involved structuring of "cleared" areas will promote faunal diversity.
- 3.: An extending diversity of animal species stimulates self-regulating processes in the agroecosystem and thus helps to stabilize the ecosystem.

Carabids occupy a special place in the group of insects, studied in this project. They often occur in high numbers of species and individuals on fields and are seen to be indicators of the quality of various environmental impacts.

Despite large field sizes and generally large-scale landscape structures, the initial situation on the until that moment conventionally managed fields of the ecofarm was characterized by broad diversities and high activity densities of carabid communities. This fact may be attributed to the principally lower intensity of arable farming in the former East Germany. However, the large number of species as observed at present on fields in the Central Germany dry region is a result of favourable climatic conditions in that area.

15-115

DISTRIBUTION OF GEMINIVIRUSES IN MONOCOTYLEDONS AND *CICADULINA* SPECIES IN ECOLOGIES IN MAIZE GROWING REGIONS OF KENYA (HOMOPTERA: CICADELLIDAE)

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The distribution of geminiviruses and *Cicadulina* species was studied in the main maize growing regions of Kenya. The diverse ecologies in the central highlands of Kenya, particularly on the eastern and southern fringes of Nyambene Hills, Mount Kenya and Aberdare Range produced the most significant data. Geminiviruses were recorded in different agro-ecological zones (AEZs). Maize streak virus (MSV) prevailed in maize planted in AEZs where mean annual soil moisture availability was above 25%; whereas MSV, panicum streak virus (PanSV) and sugarcane streak virus (SSV) infected grasses were distributed in AEZs having above 50% moisture regime. The viruses were restricted to a 300-kilometre wind corridor lying between Nyambene Hills, in the north east, where less than one percent MSV occurred, to the environs of Nairobi, in the south west, where MSV was 100% in individual fields, with most infections appearing early rather than late in each of the growing season. The spatial and temporal distribution of the geminiviruses reflected the route and times of dispersal of migrant populations of *Cicadulina* vectors.

The data (which will be presented) on the distribution of geminiviruses and vectors suggest a model where MSV is a disease of annual grasses distributed to crops during seasonal migrations.

15-116

APHID POPULATION DYNAMICS AND VIRUS SPREAD ON SEED POTATO CROPS IN ITALY

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Systematic observations were carried out in various Italian potato-growing areas over the past four years to define the best moment for haulm destruction.

Results indicated that there were great variations between localities and from year to year regarding to the numbers of aphid species, total number caught in the yellow traps and aphids counted on potato leaves. Potato virus Y was the most widespread virus, followed by Potato virus S, while Potato leaf roll virus had almost disappeared.

In the north, the high number of aphids caught and the great variations in the aphid populations made it impossible to correctly define the moment for haulm destruction. On the contrary, in the south of Italy, in the Sila Highland, where the conditions are more similar to those north of the Alps and the number of aphids and species were lower, it was easier to adopt the *Myzus persicae* and *Macrosiphum euphorbiae* count to decide the date for haulm destruction.

15-117

VARIATION OF TRANSMISSION EFFICIENCY OF SOYBEAN DWARF VIRUS AMONG LOCAL CLONES OF *AULACORTHUM SOLANI* (KALTENBACH) IN JAPAN (HOMOPTERA: APHIDIDAE)

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Soybean dwarf virus (SDV) is a pathogen of soybean dwarf which causes serious yield losses in soybeans. This virus is transmitted by the foxglove aphid, *Aulacorthum solani*, in a persistent manner. Although the vector insect is distributed commonly throughout Japan, the occurrence of SDV is restricted to the northern parts of this country, Hokkaido Island and north-eastern Honshu Island. To find a possible reason for this distributional discrepancy between SDV and the vector, we examined differences in transmission efficiency among local populations of *A. solani*. Twenty-six local clones of *A. solani* were collected from Hokkaido Island and Honshu Island which includes SDV absence regions, and their transmission efficiency of the virus was compared. All aphid clones could transmit SDV but the transmission efficiency was remarkably different among them. A clone from Naganuma in Hokkaido Island transmitted the virus most efficiently (average transmission rate = 0.65) and the transmission rate of a clone from Kurosaki in Niigata prefecture was the lowest (average transmission rate = 0.03) in the 26 clones. A geographical trend was observed on the extent of SDV transmission efficiency of the aphid clones. Almost all aphid clones from the Japan Sea side of northern Honshu Island showed lower virus transmission efficiency and, on the other hand, many clones from the Pacific Ocean side of the island attained relatively higher transmission efficiency. This geographic differences in SDV transmission efficiency among vector populations may partly explain the restricted distribution of SDV.

15-118

SPREAD OF ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) IN RELATION TO APHID POPULATIONS AND ITS TRANSMISSION BY DIFFERENT APHID SPECIES

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Zucchini yellow mosaic potyvirus (ZYMV) is transmitted by aphids in non-persistent manner and is widespread in cucurbit crops, such as: zucchini, squash, melon, watermelon. In order to understand its epidemiology we studied its spread in zucchini crops, hybrid Jedida, in relation to aphid population in Vassilika, Thessaloniki. We tested, in lab experiments, its transmission by of different apterae aphid species, such as: *Aphis nerii*, *Aphis fabae*, *Brevicoryne brassicae*, *Myzus persicae*, *Rhopalosiphum maidis* and *Rhopalosiphum padi*. These species were tested because of their abundance either in Moericke traps, in zucchini crops, and/or Rothamsted suction traps. All species tested transmitted ZYMV, although they differed in transmission efficiency. *M. persicae* was the most efficient vector whereas *B. brassicae* was the most inefficient. *M. persicae* was the most abundant species caught in both Moericke (Vassilika Thessaloniki) and suction traps (Thessaloniki, Velesino Thessalia, Kopais Boiotia). Its high incidence in conjunction with its high transmission efficiency make it an important vector of ZYMV. All the other species tested are reported for the first time as vectors of ZYMV. Despite their low transmission efficiency, compared to *M. persicae*, they may contribute to ZYMV spread in areas where they occur in high numbers.

15-119

SEASONAL FLIGHT PATTERN OF *Circulifer haematoceps* (M. ET R.) (HOM., CICADELLIDAE) IN YOUNG CITRUS ORCHARDS

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Citrus stubborn disease (CSD), caused by *Spiroplasma citri* Saglio et al., is a most serious disease in the Mediterranean and the Near East. In the east Mediterranean citrus-region of Turkey, CSD is exclusively vectored by the leafhopper *Circulifer haematoceps* (M. et R.) And apparently only young citrus is subjected to natural infection. Therefore the seasonal flight patterns of *C. haematoceps* were monitored by yellow sticky-traps in four young citrus orchards and three nurseries in the east Mediterranean region of Turkey during 1993-1995. Furthermore, interactions of the pest and the virus were examined.

The flight activity of *C. haematoceps* was characterized by a major peak in May and a second smaller peak in November. This specific flight pattern was identical at all locations, for all years and regardless to orchard age. *C. haematoceps* did not settle in the orchards but migrated from and to overwintering sites in May and November. Interestingly, the total number of *C. haematoceps* caught per year was 5-fold higher in newly established orchards compared to 3-4 year old orchards. This significant change in the flight behaviour of *C. haematoceps* may explain why only young orchards are subjected to natural infection of *S. citri*. Furthermore, the number of leafhopper vectors on yellow sticky-traps placed near ground was 20-fold higher than the number of *C. haematoceps* trapped in young trees (1.2 m height), indicating that most of the individuals move in lower strata, minimizing the risk of disease spread.

15-120

THE ROLE OF SYMBIONIN, AN APHID ENDOSYMBIONT-DERIVED PROTEIN, IN THE TRANSMISSION OF POTATO LEAFROLL VIRUS

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Luteoviruses are single-stranded RNA viruses which infect a wide range of mono- and dicotyledonous plants in which they replicate almost exclusively in the phloem tissue. They are transmitted by aphids in a circulative manner. Briefly, this implies that virus particles are ingested along with phloem sap from infected host plants and transcellularly transported through the gut into the haemocoel. The acquired virus particles are retained in an infective form in the haemolymph for the aphids' lifespan, apparently without replication. Upon contacting the accessory salivary glands, they may be transported through this gland, eventually arriving in the salivary duct from which they are excreted with the saliva during feeding of the aphid. Luteoviruses display a high degree of vector specificity at the various transmission barriers in the aphid. These well-developed specificities suggest an intimate association between a luteovirus and its vector in which both surface domains of the viral capsid and sites or substances in the aphid are involved. Recently, we have demonstrated that symbionin, a protein abundantly produced by the intracellular symbiotic bacteria of *Myzus persicae* (Sulz.), and released into the haemolymph, determines the persistent nature of PLRV in the aphid. The virus exhibits a high specific affinity for symbionin in *in vitro* binding assays. Here, we report on the identification of the protein domains of symbionin involved in virus binding.

15-122

TRANSMISSION CHARACTERISTICS OF *SPIROPLASMA CITRI* AND ITS EFFECT ON LEAFHOPPER VECTORS FROM THE *CIRCULIFER TENELLUS* COMPLEX

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Several leafhopper variants of the *Circulifer tenellus* complex were collected in "stubborn" affected areas in Israel. Two of these variants transmitted the disease agent to *Matthiola incana* after being injected with *Spiroplasma citri*. The variant from *Atriplex halimus* was collected in the Jordan Valley and designated *Circulifer tenellus*-A (CTA). The other leafhopper variant reproduced on *Portulaca oleracea* and been collected both in the Jordan Valley and in the Coastal Plains and designated *Circulifer tenellus*-P (CTP). Transmission characteristics were determined for both variants. A high rate of transmission (43.3%) was obtained by single leafhoppers of the CTA variant that were injected with the Amiad *S. citri* isolate deriving from the Upper Galilee, compared with 7% transmission that were obtained by the CTP leafhoppers. The *S. citri* isolate from Gilgal in the Jordan Valley, was not transmitted by either the CTA or the CTP leafhopper variants. Injection was found to be more effective than acquisition access feeding to render the leafhoppers infective either for the CTA or the CTP leafhoppers. The minimum acquisition access period (AAP) needed for the CTA variant to transmit the Amiad isolate was 1 hr. Longer AAPs did not always result in a higher rate of transmission. The minimum incubation period was 6 days and the time required for 50% of the population to acquire the pathogen (=LP₅₀) was 26 days. The minimum inoculation access period (IAP) was 1 hr. After an IAP of 5 days 54.1% of the plants were infected. We failed to determine the same transmission parameters for the CTP variant, as no transmission was obtained even when groups of 5-6 insects were placed on a single plant. The significance of these findings in the disease epidemiology in the Near East, is discussed.

15-121

COMPARATIVE EPIDEMIOLOGY OF THREE MAIZE VIRUSES IN REUNION ISLAND IN RELATION TO THE POPULATION DYNAMIC OF THEIR VECTORS, *CICADULINA MBILA* AND *PEREGRINUS MAIDIS*

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The simultaneous presence of maize streak virus (MSV), maize stripe virus (MSpV) and maize mosaic virus (MMV) in Réunion has enabled us to study their comparative epidemiology.

Symptomatological ratings were taken through three years of culture on weekly sowings of the temperate hybrid INRA 508 and the composite variety IRAT 297.

Fluctuations of emigrant populations of *C. mbila* and *P. maidis* and climatic factors (temperature, rainfall and relative humidity) were measured and analysed by time series and stepwise regression analyses. Streak is the dominant disease, particularly during the warm rainy season and MMV was less frequent. Highest autocorrelations were observed with a time-lag of 12 months, confirming the annual periodicity of the fluctuation.

Pattern of change of insect numbers was positively correlated with the change in disease incidence (correlations ranging from 0.65 to 0.87). Disease incidence and vector numbers always remained constant or increased slowly with temperature up to 24°C and increased rapidly above 24°C. The relationship between rainfall, relative humidity, disease incidence and vector numbers is less clear.

Depending on the series, from 63% to 80% of the variance of disease incidence was explained by the stepwise regression with vector numbers, and (sometimes) temperature, rainfall or relative humidity. Therefore, the close adjustment found between observed and data calculated with the resulting regression allowed us to propose a simple epidemiological model.

Epidemiological data especially from Africa are compared in order to better understand the epidemiology of these viruses. Further work is needed to confirm the validity of the model. The rate of plants infected by the three viruses and the number of *P. maidis* per plant were significantly lower ($P = 0.0001$) on IRAT 297 than on INRA 508. The resistance behavior of IRAT 297 under natural conditions points to its utilization as resistance donor in breeding program geared toward obtaining multi-resistance maize genotypes.

15-123

THE OCCURRENCE OF CITRUS THRIPS, *SCIRTOTHRIPS AURANTII* FAURE IN MANGO ORCHARDS IN SOUTH AFRICA AND THE RESULTING IMPLICATIONS FOR PEST MANAGEMENT

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The citrus thrips, *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) is the most important thrips species found on mangoes in South Africa. *S. aurantii* causes lesions on the fruit and render it unsuitable for the export market. Leave malformation and stunting of new growth is also caused. In order to control *S. aurantii* effectively on mangoes, population levels should be monitored regularly. The objective of this study was to determine when *S. aurantii* occurred in abundant numbers in mango orchards, when fruit were infested and when lesions were caused.

Weekly monitoring was conducted in different localities in the Mpumalanga Province from 1992 - 1995. *S. aurantii* were monitored by means of yellow card traps and by counting thrips on the fruit. The percentage fruit displaying lesions were annotated and fruit size was also measured.

Monitoring *S. aurantii* by means of card traps and fruit counts were effective and were correlated with fruit displaying lesions. High numbers of *S. aurantii* were present when mango fruit were small and during flushing periods. Results indicated that lesions were caused from fruit set and highest number of thrips were present when fruit were between 20 - 50 mm in length. Towards the end of the fruiting period, virtually none were present on the fruit. It is therefore important for producers to do regular orchard inspections from fruit set until the fruit are approximately 50 mm in length.

15-124

BIOLOGICAL STUDY ON WHEAT THRIPS

HAPLOTHRIPS TRITICI KURD. (THYS. PHLAEOTHRIPIDAE)

IN TEHRAN PROVINCE, IRAN

BY: P. AZMAYESH FARD

ENTOMOLOGY DEPARTMENT COLLEGE

OF AGRICULTURE TEHRAN UNIVERSITY KARAJ-IRAN

Biological researches on wheat thrips in Tehran province revealed that *Haplothrips tritici* Kurd. attacks wheat and barley fields. Its life cycle includes 2 nymphal 1 prepupal, 2 pupal and adult stages which are easily separated by the descriptions presented. The female insects appear in early May and feed on host leaves, mostly terminal ones, but after heading they all move to ears. It seems that parthenogenesis to be a common way of reproduction in this species. The eggs are laid in 2-15 batches in spikes exactly beneath the glumes. A female which has a preovipositional period of one month, lays 130-5 eggs during the life time. The developmental time of different stages were determined in laboratory and field.

Population dynamic and seasonal variability of all stages of the insects are also studied. The insect overwinters as second instar nymphs in soil at the depth of 5-10 cm or inside the wheat stubbles.

15-125

POPULATION PARAMETERS OF REPRODUCTIVE BIOTYPE IN *THRIPS TABACI* (THYSANOPTERA: THRIPIDAE)

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It is well known that parthenogenetic reproduction occurs in many thrips species. Both arrhenotokous and thelotokous parthenogenesis occurs in *Thrips tabaci*. Both reproductive biotypes were found in Shimane and Miyagi of Japan. Population parameters of those biotypes were compared. There was no difference in developmental duration and a big difference in produced egg number between two biotypes. Sex ratio of arrhenotokous type was 0.73. Therefore, arrhenotokous type produced females as same as thelotokous type. Parameters of population increase of arrhenotokous and thelotokous type were as follows; Mean generation time was 17.7 and 21.1, Net-reproduction rate (R_0) was 79.3 and 78.4, intrinsic rate of population (r_m) was 0.2387 and 0.2704, respectively.

Arrhenotokous type could be distinguished from thelotokous type by esterase zymogram of every stages of thrips. There were eight bands on esterase zymogram of *T. tabaci*. Each biotype has characteristic band, No. 7 in arrhenotokous and No. 6 in thelotokous.

15-126

A TRIAL OF WINTER REGIONAL CONTROL FOR *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) (THYSANOPTERA: THRIPIDAE) IN JAPAN.A. Tataru, K. Imigi¹, T. Sagisaka¹

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F. occidentalis invaded to Japan in 1990. Since that, this species has become one of major pest of mandarin orange and vegetables in greenhouse and flowers in commercial production in greenhouse and field in Shizuoka prefecture. Since more than 200 species of plants have recorded as host plant for *F. occidentalis* (Bryan and Smith, 1956) and the thrips bore the impact of low temperature, nursery stocks of cultivating chrysanthemum and many winter annual weeds around the field and the greenhouse were suitable overwintering places for *F. occidentalis*. The overwintering populations rapidly increase in early spring, causing severe damage of flowers, strawberry and Chinese-lantern plant in Shizuoka. To oppress the occurrence in spring, a trial of winter regional control experiment was done in 100 ha of the chrysanthemum cultivation zone in Hamamatsu, Shizuoka in 1993. The recommended regional control program was as follows.

- 1) Remove extra chrysanthemum stocks till late January.
- 2) Burn the leavings of chrysanthemum or bury them into soil.
- 3) Remove the all flowers from the nursery chrysanthemum stocks.
- 4) Remove weeds around the fields and the greenhouses till late January.
- 5) Spray pesticide to the nursery stocks, flowers in field and greenhouse, and flowers in home gardens at the same time, on February 1 and February 8.

Density of *F. occidentalis* in control area after above treatments was lower than uncontrolled area. However, it is considered that more pesticide treatment in March is necessary because the density of *F. occidentalis* in control area in early March became to be high from early February.

15-127

THRIPS *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) OF ROSES IN GREENHOUSES IN EASTERN PART OF CROATIA

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The western flower thrips *Frankliniella occidentalis* (Pergande) is permanent cause of the products problem. This cause is present on the roses and weeds (*Stellaria media* (L.) Vill., *Convolvulus arvensis* L.) which are in the greenhouses in the eastern part of Croatia.

The investigation was carried out in the greenhouses "Orhideja" Magadenovac, where the main products are roses. They have had problems on roses since 1990 and this pest is present in every production. Many cultivars and weeds that are surrounding the greenhouses are good hosts for *F. occidentalis*. The presence of the plants suitable for the thrips food permits the easily developing of the thrips.

Control measures were provided by insecticides: Karate 2,5 EC, Dilon 50 EC, Lannate L, Evisect S and Vertimec EC. All insecticides reduced population of the thrips. Vertimec EC gave the best results and population was reduced by 85%.

The population of the thrips on the roses in the greenhouses is successfully performed by the profilactical and the chemical control measures.

15-128

FRANKLINIELLA SCHULTZEI (THYSANOPTERA: THIRIPIDAE) ASSOCIATED WITH TOMATO SPOTTED WILT VIRUS INCIDENCE IN SOLANACEOUS VEGETABLES FROM NEW SOUTH WALES AUSTRALIA

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Both *Thrips imaginis* Bagnal and *T. tabaci* Lindeman were found associated with seed potato crops in the Crookwell district (-34° 28', 149° 29'; altitude 700 m), 1990-93, but only *T. tabaci* transmits Tomato Spotted Wilt Virus (TSWV). TSWV incidence was below 0.1%. A wider survey throughout NSW in 1994 identified three potato crops infested by *Frankliniella schultzei* (Trybom) with 50-100% infection levels. During petroleum spray oil (PSO) field experiments on processing tomatoes at Leeton (-34° 33', 146° 24'; altitude 140 m) during the 1994/95 season, *F. schultzei* was associated with between 16 and 80% infection with TSWV in tomatoes and capsicums, depending on the effectiveness of insecticide use. High volume, over 1,000 L/ha, of 1% PSO restricted the incidence of TSWV to less than 20% of plants. There was an exponential increase in TSWV incidence with increasing density of thrips.

These observations emphasise the potential for a migratory thrips like *F. schultzei* to infect a high proportion of crops over a wide area with TSWV.

15-130

FACTORS CONTRIBUTING TO INCREASES OF BEMISIA TABACI (GENN.) POPULATIONS: DEFINING THE OUTBREAK PYRAMID (HOMOPTERA: ALEYRODIDAE)

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The rise in status of *Bemisia tabaci* as a world-wide pest of agriculture and floriculture has accelerated over the past 15 to 20 years. Explanations for this rise have been varied, but in many cases have implicated the pervasive use of insecticides as a major contributing factor. Resistance to insecticides has been documented in many crop situations and countries, although the connection between the identification of resistance (often based on laboratory-defined resistance through comparisons to inbred reference strains) and loss of field control has not always been clear. Other insecticide-related effects such as depredation of natural enemy populations and/or hormoligosis due to sub-lethal pesticide residues may also have contributed to *B. tabaci* outbreaks.

Three consecutive years of monitoring responses of *B. tabaci* populations to insecticides in the Imperial Valley, CA failed to detect progression to higher resistance levels. Bioassay data indicated instead that susceptibility to most insecticides increased during this period, while insecticide field trials demonstrated good field efficacies for many insecticide treatments against whiteflies. Natural control has been inadequate even in organically-grown crops, and there has been no indication of accelerated population growth due to hormoligosis. However, whitefly populations still have grown to outbreak proportions, demanding a more comprehensive consideration of factors that contribute to *B. tabaci* outbreaks other than just insecticide-related factors.

The outbreak pyramid is a conceptual presentation of four principle components that contribute to whitefly outbreaks. At the base of the pyramid is (1) climate, the most fundamental and unalterable component, which can accelerate whitefly generation times to 2.5 weeks under hot, rainless conditions. Built upon the climate base is the (2) agriculture, the types of crops, their sequences and relative acreages that determine the quality of the resource base available for whitefly populations to exploit. The third component is (3) biotic, or the intrinsic potential of *B. tabaci* to grow and increase its numbers within its environment. At the top of the pyramid is the (4) management component, which may not be adequate to contain *B. tabaci* populations depending on the collective potential contained within the bottom three levels of the pyramid. It is argued that a lush agricultural system in a hot, arid climate can increase *B. tabaci* populations at a rate whereby they escape their natural enemies, and, in some cases, is beyond the economic and/or practical feasibility of management inputs to suppress whitefly populations below economically damaging levels.

15-129

MONITORING GEMINIVIRUS ACQUISITION AND TRANSMISSION BY WHITEFLIES IN ARTIFICIAL FEEDING CHAMBERS USING POLYMERASE CHAIN REACTION

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The ability of adult whiteflies to ingest squash leaf curl virus (SqLCV) from infected plants was monitored by polymerase chain reaction (PCR) using an artificial feeding assay. *Bemisia tabaci* (vector) and *Trialeurodes vaporariorum* (non-vector) adult whiteflies were allowed an acquisition access period (AAP) of either 2, 8, 24 and 48 h on healthy or SqLCV-infected pumpkin, and a 24 hr (simulated) inoculation access period (IAP) on sucrose feeding substrate in artificial feeding chambers. The presence of SqLCV DNA was monitored by PCR in plant extracts, whole body whitefly extracts, sucrose feeding substrate containing saliva, and honeydew. PCR primers that amplify a 550 bp fragment of the SqLCV coat protein gene were used in PCR assays to ascertain virus presence. Virus was detectable in adult whiteflies fed on SqLCV-infected plants and in infected plants, but not in non-viruliferous whiteflies or in healthy plant controls. SqLCV DNA was also detectable in saliva/sucrose, honeydew, and whole body extracts of *B. tabaci*, allowed 8-48 hr AAP's with 24 hr IAP on sucrose. However in *T. vaporariorum*, viral DNA was detected in honeydew and whole body extracts, but not in the saliva/sucrose. This study provides the first direct evidence of geminivirus in vector whitefly saliva, and demonstrates that some form(s) of virus/viral DNA is (are) ingested, and passed through, both vector and non-vector adult whiteflies, given a sufficient AAP.

15-131

ASSAYS FOR WHITEFLY (HOMOPTERA: ALEYRODIDAE) RESISTANCE IN MELON, *Cucumis melo*

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Whiteflies cause much damage to vegetable and row crops in several countries. The problem has been exacerbated in recent years by the presence of a recently described species, *Bemisia argentifolii* (also called strain-B *Bemisia tabaci*). Host plant resistance in melon and other vegetables is a desired, but under accomplished, management tool against whiteflies. In developing a resistance breeding program, having a rapid and reproducible method for screening germplasm for resistance to whiteflies is essential.

Multiple assays were conducted in field, greenhouse, and laboratory conditions for evaluating melon germplasm for resistance to *Bemisia argentifolii*. Field tests with many melon entries were conducted in southern California and Arizona where high natural infestations occurred. The more promising resistant field entries, based on plant condition and plant size, were evaluated in greenhouse and laboratory tests, respectively. Plant growth response (biomass, size and vigor) to infestation, and whitefly attractiveness to melon entries for feeding and oviposition were examined within two weeks after plant emergence. Survival from egg to adult emergence was determined on live plants. Entries were compared with 'Top Mark,' a susceptible commercial entry.

15-132

DETECTION OF GEMINIVIRUS IN THE WHITEFLY *BEMISIA TABACI* (GENNADIUS) (HOMOPTERA: ALEYRODIDAE)W.B. Hunter, E.Hiebert¹, S.E.Webb², J.E. Polston³, J.H. Tsai⁴

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Detection of geminiviruses in the whitefly vector, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) B-biotype, was examined by light, scanning- and transmission electron microscopy, fluorescent and confocal laser microscopy to elucidate the location and movement of virus within whiteflies. Two different geminiviruses, tomato mottle virus (TMOV), and a cabbage geminivirus were examined. Geminiviruses were detected by indirect fluorescein conjugated antibody technique in the anterior region of the midgut, filter-chamber, and primary salivary glands of whiteflies. These are most likely the sites where virus passes through membranes before transmission can occur. Virus detection was low for both geminiviruses (3-20%). The difficulty of detecting virus after insects fed on healthy plants suggests that virus titers were low within individuals under these conditions, which may be due to a lack of virus replication within the vector. A model for Virus movement during acquisition is discussed.

15-134

DAMAGE ON "HASS" AVOCADO LEAVES, WEBBING AND NESTING BEHAVIOR OF *OLIGONYCHUS PERSEAE* TUTTLE, BAKER AND ABBATIello (ACARI: TETRANYCHIDAE)

1

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The damage of *Oligonychus perseae* Tuttle, Baker and Abbatiello on avocado trees occurs mainly on the underside of the leaves along the midrib, main veins and leaf depressions. the lower epidermal, spongy parenchyma and palisade parenchyma cells of the leaf tissues are destroyed. Large necrotic areas on the underside of the leaf result from feeding by high population levels of the mite. Feeding and reproduction takes place in "nests" of silken webbing, which also provide protection from some predator mites and other natural enemies. *O. perseae* shows a modification of the life type web-nest (WN-c). The highest number of nests built/female was 12.17 at 20 °C, and the highest number of eggs/female/nest was 5.20 at 25 °C

15-133

BIOLOGY, LIFE TABLE AND MATING BEHAVIOR OF *OLIGONYCHUS PERSEAE* TUTTLE, BAKER AND ABBATIello (ACARI: TETRANYCHIDAE)

1

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The biology of *Oligonychus perseae* Tuttle, Baker and Abbatiello was studied at four different temperatures on excised "Hass" avocado leaves (*Persea americana* Mill.). The time required for development from egg hatch to adult was : 34.89, 16.90, 13.8 and 9.81 days, at 25, 20, 25 and 30 °C, respectively. The highest fecundity (45.80 eggs/female) occurred at 25 °C, and the highest oviposition rate (1.84 eggs/female/day) was at 30 °C. The highest mortality for development was 32.30 % for the combined stages at 30 °C. The theoretical lower threshold temperature for development was 7.80 and 9.00 °C, for the combined stages of female and male, respectively. The thermal constant for completion of development was 200 and 166 day-degrees for the female and male, respectively. The sex ratio ranged from 1.82:1 to 239:1 (female:male). The calculated intrinsic rate of increase was highest (0.1440) at 30 °C. The net reproductive rate was highest (25.80) at 25 °C. The shortest mean generation time (18.38) occurred at 30 °C.

15-135

SOME BIOLOGICAL AND MORPHOLOGICAL DIFFERENCES BETWEEN GALL AND VAGRANT FORMS OF *PHYTOPTUS AVELLANAE* NAL.(ACARINA: ERIOPHYOIDEA)

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Big bud mite (*Phytoptus avellanae* Nal.), which is one of the most important pests of hazelnut, has two forms, gall and vagrant. The gall form of *P. avellanae* lives in big buds. It has a simple life cycle and a single nymph type. The vagrant form of *P. avellanae* lives on vegetative and generative organs and in big buds. The latter has a different and rather complex life cycle and two nymph types.

In this study gall and vagrant forms of *P. avellanae* were examined biologically and morphologically. Biological studies were performed at 20±1°C, 24±1°C and 70 % R.H. in the laboratory and mites were reared on leaf discs and duration of the different stages were determined. Gall form can not survive on the leaf disc after the first instar nymph stage while the vagrant form lives on the leaf disc. Morphological comparisons showed that gall and vagrant form were significantly different from each other in size, shape and colour.

15-136

INFESTATION PROCESS OF LOQUAT RUST MITE (TENTATIVE NAME) *ACERIA* SP. ON LOQUAT FRUIT

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Loquat rust mite (tentative name), *Aceria* sp. is one of the important pests of loquat in Japan, especially in growing in green house. It widely distributes at loquat cultivated areas in China and Japan. Host plant is restricted only loquat, *Eriobotrya japonica*, including almost of the cultivated and wild varieties. This pest cannot live on other *Eriobotrya* species, for example *E. deflexa*, etc. It inhabits inside of flower cluster and other sheltered part of bud and base of leaf, and causes so-called **Tateboya** symptom, brownish injury of fruit surface from pedicel to apex. In Japan damage occurs especially in green house, because rust mite is able to increase rapidly by suitable temperature for development. After standing of flower bud from August, it increases inside of flower cluster and attacks on surface of young fruit. **Tateboya** symptom occurs just before the bagging of fruit. This damage can be lightened by one time of spray of acaricide, for example **Pyridaben**, etc or fungicide **Thiophanate-methyl** during flowering period of loquat.

15-138

MITES ASSOCIATED WITH CULTIVATED MUSHROOMS IN TÜRKİYE

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The world acarofauna of cultivated mushrooms is extremely rich. In the studies were made in Türkiye nine harmful and five beneficial mite species were determined. Harmful species were *Pygmephorus allmanni* Krczal, *P. madanlarae* Ramaraju and Madanlar, *P. sellnicki* Krczal, *P. smileyi* Ramaraju and Madanlar, *P. turkiensis* Ramaraju and Madanlar (Prostigmata, Pygmephoridae), *Caloglyphus berlesei* (Michael), *C. rhizoglyphoides* (Zachvatkin), *Mycetoglyphus fungivorus* Oudemans (Astigmata, Acaridae) and *Histiostoma sapromyzae* (Dufour) (Astigmata, Histiomidae). Beneficial species were *Eugamasus butleri* Hughes (Mesostigmata, Parasitidae), *Poecilochirus belkahvensis* Ramaraju and Madanlar, *P. hyatti* Ramaraju and Madanlar, *P. simpliseta* Ramaraju and Madanlar and *P. torbaliensis* Ramaraju and Madanlar (Mesostigmata, Parasitidae). In addition to these beneficial species, some unidentified mites took part in the genus *Arctoseius* (Mesostigmata, Ascidae), the subfamily Laelapinae (Mesostigmata, Dermanyssidae), and the families Ascidae, Digamaselidae, Macrochelidae, Parasitidae (Mesostigmata), Anystidae and Cunaxidae (Prostigmata).

15-137

A NEW SIMPLIFIED METHOD TO ESTIMATE POPULATION DENSITY OF THE RUST MITE (*ERIOPHYES CHIBAENSIS* KADONO) ON JAPANESE PEAR USING STICKY TAPES AND WATER-SENSITIVE PAPERS

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To estimate the population density of the rust mite (*Eriophyes chibaensis* KADONO) on Japanese pears invisible to the naked eye because of its minuteness, a new simplified method using sticky tapes and water-sensitive papers (Spraying Systems Co.) was developed. The procedures for investigation are as follows.

1. Expanded leaves on the top of elongated shoots were cut into two parts along midrib by knife in May to June.
2. Sticky transparent tapes were pasted on the back of the pieces of leaves.
3. These sticky tapes were peeled from the pieces of leaves and pasted on the water-sensitive papers with strongly rubbing their surface by the finger nail.
4. The parts of crashed mites on the water-sensitive papers were colored blue.
5. The density of the colored spots on the water-sensitive paper was graded as the index of the population density of mites by compared with the practical mite density observed by stereomicroscopy in advance.
6. This method was considered to be useful and effective enough to estimate rust mite populations.

15-139

BIOLOGICAL OBSERVATIONS ON PHYTOSEIID MITES (PARASITIFORMES, PHYTOSEIIDAE) LIVING ON HAZELNUT (*CORYLUS AVELLANA* L.) AT TWO DIFFERENT ALTITUDES IN SICILY

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Surveys on phytoseiid mites living on hazelnut (*Corylus avellana* L.) at two different altitudes (m 500 a.s.l. and m 1000 a.s.l.) were carried out during 1991- 1993. Nineteen species of phytoseiids were recorded as a whole. Among them 8 species (*Kampimodromus aberrans* (Oud.), *Typhlodromus intercalaris* Liv. & Kuzn., *Typhlodromus cryptus* Athias-Henriot, *Typhlodromus rhenanoides* Athias-Henriot, *Amblyseius andersoni* (Chant), *Euseius stipulatus* (Athias-Henriot), *Paraseiulus erevenicus* Wainst. & Arutun., *Phytoseius finitimus* Ribaga) were present in both fields, and the first four were the most frequent. *T. intercalaris* was the dominant one (53%) at m 500 a.s.l., followed by *K. aberrans* (34%), *T. rhenanoides* (8%) and *T. cryptus* (6%), while *K. aberrans* was the dominant one (60%) at m 1000 a.s.l., followed by *T. cryptus* (27%), *T. intercalaris* (13%) and *T. rhenanoides* (1%), showing the preference of *K. aberrans* for higher altitudes and lower temperatures. Moreover the population increased one month earlier at m 500 a.s.l. than at m 1000 a.s.l. Furthermore the distribution of phytoseiids on the plants was also ascertained. For this purpose the trees were divided into three parts: trunk, low branches and tall branches. On the whole the various species did not show any preference for any of the three different parts of the trees as they were uniformly distributed on the trees during the observation periods.

15-140

THE EFFECTS OF PESTICIDES AND OTHER VARIABLES ON PHYTOSEID MITES (PARASITIFORMES, PHYTOSEIIDAE) IN VINEYARDS OF THE REGIONS CAMPANIA AND LAZIO

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Investigations on phytoseid mites have been carried out between 1992 and 1995 in 26 vineyards in Campania and 22 vineyards in Lazio. During the four years of observation, pesticide control, grape-vine cultivar, vineyard age, cultivation methods and species of nearby plants were registered in each of the 48 vineyards. The population dynamics of phytoseid mites in relation to thermo-hygrometric variations was also noted in several vineyards. The following eight species, listed in decreasing order according to their presence, were found in the vineyards of Campania: *Kampimodromus aberrans* (Oudemans), *Typhlodromus exilaratus* Ragusa, *Amblyseius andersoni* (Chant), *Phytoseius finitimus* Ribaga sensu Denmark (1966), *Typhlodromus criptus* Athias-Henriot, *Amblyseius stipulatus* Athias-Henriot, *Seiulus soleiger* Ribaga, *Typhlodromus kerki-rae* Swirski & Ragusa. The following seven species were found in Lazio: *T. exilaratus*, *P. finitimus*, *K. aberrans*, *A. andersoni*, *Amblyseius californicus* (Mc Gregory), *T. criptus* and *Seiulus amaliae* (Ragusa & Swirski).

It has been noted that, amongst the external elements, pesticide control has assumed a key role on the density of the populations of these beneficial Arthropods causing the total disappearance of phytoseid mites in some vineyards where non-selective pesticides have been used over and over again.

Other external components have affected the phytoseid populations to a lesser extent: cultivars, temperature, humidity, wild and cultivated plants growing near the vineyard.

The method of cultivation, the agronomic practices and the age of the vineyard have no influence on the density of phytoseid populations.

15-141

PEST MITES ON CULTIVATED EDIBLE MUSHROOMS IN JAPAN

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Increasingly, several species of mites are becoming serious pests to relatively new cultivation of indoor mushrooms in Japan. Mushroom pest mites have been investigated regularly since 1990. These investigations have concluded that most species recorded belong to Acaridae, Histiogastromatidae or Pygmephoridae.

These mites appeared while mushrooms were being cultivated under temperatures of 18 to 23°C with humidity higher than 80% R.H. In most cases, weed fungi such as *Trichoderma* spp. coappeared in bottles of mushroom cultivation with mites. Under laboratory conditions, *Tyrophagus putrescentiae*, the species recorded most frequently, is observed to excrete more than 10 spores of a weed fungus per a fecal pellet. Because approximately half of the spores are germinative, it is suggested that mites carry these fungal spores into bottles. As control measures, only a temporary stopping cultivation combined with fumigation have achieved success so far.

15-142

THE INCIDENCE OF BROADMITE (*Polyphagotarsonemus latus*) (BANKS, 1904) (ACARI: TARSONOMIDAE) IN NINE BEAN GENOTYPES, IN GREENHOUSE CONDITIONSM.A.G. ORIANI¹, F.M. LARA²

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This research intended to evaluate the incidence of broadmite, *Polyphagotarsonemus latus* (Banks), on nine bean genotypes, mainly those that present arcelin. This protein could be associated with the resistance to bruchids, *Zabrotes subfasciatus* (Boh.) and *Acanthoscelides obtectus* (Say). The tests were carried out in greenhouse conditions, with the genotypes: Arc 3s, Arc 5s (wild genotypes with arcelin); Arc 1, Arc 2, Arc 3, Arc 4 (genotypes with arcelin in a breeding program) and Porriolo 70, Bolinha, IAPAR MD 808 (commercial genotypes without arcelin). These genotypes were planted in pots, with two plants per pot and ten repetitions, in dry and wet season. Three leaflets per pot were collected from the top leaves when the plants were 24 day-old. The counting of mobile forms of mites present in the lower surface of the leaflet was carried out in laboratory. In the wet season, the incidence by broadmite was significantly lower on Arc 3s and higher on Porriolo 70 and Bolinha genotypes. In the dry season, Bolinha, Arc 2 and Arc 1 genotypes had the biggest incidence, while the Arc 5s had the smallest incidence.

15-143

EGG MORTALITY OF THE WHITEBACKED PLANTHOPPER, *SOGATELLA FURCIFERA* HORVÁTH (HOMOPTERA: DELPHACIDAE) ASSOCIATED WITH THE FORMATION OF WATERY LESION AT OVIPOSITION SITES ON RICE PLANTS

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Association of the physiological egg mortality of *Sogatella furcifera* with the rice plant response of forming watery lesions at oviposition sites was revealed: egg survival rate dropped to less than 20% in the watery lesion within 2 days after oviposition, while 88.8% of eggs developed to the eye-spot formation stage in the non-watery lesion, on Japonica rice plants 8 weeks after planting. *S. furcifera* eggs laid in large tillers and in the main stem suffered higher physiological mortality than those laid in small tillers on the same plant. The physiological egg mortality increased steadily with progression of the tillering of plants until 10 weeks after planting. Egg density did not affect the mortality. Benzyl benzoate was isolated from the watery lesions. Water solution of benzyl benzoate exhibited ovicidal activity against *S. furcifera* eggs at concentration of ≥ 6.4 ppm. This ovicidal substance was not detected from intact rice plants, *S. furcifera* eggs or non-watery ovipositional lesions.

Newly discovered ovicidal response of rice plants explains annual fluctuations in the population growth rate of *S. furcifera* in paddy fields.

15-144

ARE THE LOPHOPIDAE (HOMOPTERA, FULGOROMORPHA) A PARAPHYLETIC TAXON?

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Of the twenty families of Fulgoromorpha currently recognised, the Lophopidae is one of the smallest with around 140 described species mainly confined to the Asian and African regions. Three genera are known from South America. The Lophopids is an important family not only from an applied aspect (*Pyrilla* and *Zophiuma* are respectively important pests on sugarcane and coconuts) but also from a fundamental point of view : Lophopidae and its proposed sister group Eurybrachidae are considered as the most recent major division within the Fulgoromorpha (Emeljanov, 1990).

In 1930, Muir proposed a new classification of the Lophopidae genera, based on metatarsal and metatibial characters. This rearrangement was stated by Muir to be "more natural" than the previous arrangements given by Melichar (1903) and Baker (1925). Since then, no improvement has been made to this classification and the monophyly of the different taxa proposed by Muir still requires validation. In the frame of a revision of the Lophopidae family (morphology, phylogeny and biogeography) using the cladistic methodology, the supposed essential characters used by Muir (1930) have been re-examined.

High magnification using microscope observation coupled with SEM studies shows :
• the apical tibial spine characters can not be used to separate *Ptuambara*, *Bisma*, and *Hesticus* from the *Menosca* group ;
• *Elasmoscelis* shows an obvious autapomorphic pattern of the particular metatarsal plate involved from the Lophopini sensus Metcalf (1955) which are then probably paraphyletic. This genus presents also an autapomorphic specialized distal tibial plate ;
• *Lucasa* cannot be separated from the remaining Lophopini genera.

At the family level, those particular characters can also be observed in several other Fulgoromorpha genera (e.g. Tropicuchidae : Huang & Bourgoin, 1991) and particularly in Eurybrachidae. If we consider that the discriminant value of the three characters (width of the vertex, lateral clypeal carinae and width of the frons) used to separate Eurybrachidae from Lophopidae is not exclusive, we conclude on the probable paraphyly of both families Eurybrachidae and Lophopidae.

15-146

MATING BEHAVIOR AND COPULATORY MECHANISM IN *HYALESTHES OBSOLETUS* SIGNORET (HEMIPTERA, FULGOROMORPHA, CIXIIDAE)

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In palearctic region, *Hyalesthes obsoletus* Sign. is known to be a vector of stolbur phytoplasma, an important plant pathogen. In France, it is involved in transmission of « bois noir » disease occurring in vineyards (Sforza *et al.*, 1996 in prep.). *H. obsoletus* is a monovoltine species, present at adult stage in eastern and southern France from June to August.
Before mating, we observed a characteristic male dance close to female by an intense fluttering of wings periodically repeated. An acoustic communication between male and female participates probably to this mating behavior. During mating, adults are in an opposited and head to foot position. In contrast to the other genus of Cixiidae, we confirmed that male's gonostyli grip the female's gonapophyses VIII by their inner side, as previously observed by Hoch and Remane (1985). In this species, a triple hooking assures maintenance of copulatory position.
An adapted staining allowed us to observe the vagina organized in different lobes. In this manner, aedeagus and two hooked-like endosomal processes are maintained. Presence of those strongly sclerotized processes into female's exit ducts is in relation with strongly sclerotized plates of the vagina wall. According to our observations, spermatheca is divided into five parts. As described in other Fulgoromorpha (Bourgoin, 1993), sclerotized ornamentations are present on bursa copulatrix ; a glandular role of these ornamentations may be advanced, either as a trophic assistance for spermatozoa or as a destruction of spermatozoa which are in excess.

15-145

STUDIES ON THE OCCURRENCE AND PREDICTION OF *MYRMELEOTETTIX PALPALIS* (ZUBOVSKY)

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The life history, habits, and ecological factors affecting occurrence of *myrmeleotettix palpalis* were studied in Xiahe County, Gansu Province in 1989—1994. The results showed that the pest had only one generation a year. The development of the nymphs undergoes about 70 days, and the longevity of the adults lasts for 64 days. In the natural temperature, the developmental zero of the first instar was 4.17°C, the second instar 4.85°C, the third instar 7.98°C, the fourth instar 8.70°C, and the fifth instar 9.00°C, and the effective temperature summation was 24.66 day degrees in the first instar, 40.13 in the second instar, 23.84 in the third instar, 36.59 in the fourth instar and 59.12 in the fifth instar.
Two linear regression equations expressing the close relationships between the occurrence number in the second ten days of May and precipitation or thermo—precipitation coefficient in the first ten days of May were established. In addition, an equation expressing the relationship between the peak period and the mean temperature in the second ten days of May and winter lowest temperature was proposed.

15-147

BEHAVIOURAL ASPECTS OF A NEW SPECIES OF *HILDA* KIRKALDY (HEMIPTERA:TETTIGOMETRIDAE) FROM THE DRAKENSBERG MOUNTAINS OF SOUTH AFRICA

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This *Hilda* sp. nov., with the proposed name *proteacola* Bourgoin, is only the second recorded species within the subgenus *Prodhilda* Bourgoin 1988. It seems to be endemic to the central parts of the Drakensberg mountains in the province of KwaZulu-Natal, between 1480-1950 metres above sea level, on plants of the genus *Protea* L. (Proteales: Proteaceae). I found it regularly on both *Protea caffra* Meisn. subspecies *caffra* Chisumpa & Brummitt and *P. roupelliae* Meisn. subsp. *roupelliae*, but only occasionally on *P. dracomontana* Beard.
Up to four generations per year occurred on *P.r.roupelliae* (between September-June) and three on *P.c.caffra* (September-May). Generations were not strictly synchronized, with all three stages (egg-nymph-imago) co-occurring on the same part of a plant. They all preferred fresh-growing plant tissues. On *P.c.caffra* and *P. dracomontana*, eggs were deposited in batches of one to several dozens mostly on the outermost involucral bracts of the buds of inflorescences and the apical end of the adjoining flowering branches, but occasionally on young vegetative shoots. On *P.r.roupelliae*, egg batches were smaller and laid only on young leaves.
All 3 life stages were regularly attended by ants. An unidentified *Myrmecaria* sp. (Myrmecinae) was the most common one at all altitudes. *Anoplolepis custodiens* (F. Smith, 1858) (Formicinae) was dominant only between 1500 -1580 m a.s.l., while two further Myrmecinae species were sometimes seen together with *Hilda* (*Prodhilda*) *proteacola* Bourgoin: a *Pheidole* sp. (probably *megacephala*) and *Crematogaster* sp. A. The proportion of ants to plant-hoppers (nymphs and/or adults) was about 3 on *P.r.roupelliae* and 5 on *P.c.caffra*, with *A. custodiens* usually having higher densities.
No apparent damage to any of the affected parts of the proteas was noted. I did not observe any predation or parasitism in this *Hilda* species, however, the very alert imagines could successfully evade a number of likely predators or parasitoids.

15-148

LEAFHOPPERS, PLANTHOPPERS AND PHYTOPLASMAS
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Knowledge on biodiversity among phytoplasmas detected either in Hemiptera or in plants are restricted to phytoplasmas causing damage to culture. Phytoplasmas are mainly transmitted by Hemiptera Cicadomorpha and Fulgoromorpha, except for some Sternorrhyncha Psyllidae. During 1995, the aim of our weekly trapping in french vineyards (Bourgogne and Rhône-Alpes) was to determine a vector of the french grapevine yellows, called « bois noir », whose pathogenic agent belongs to the stolbur group. 2400 Auchenorrhyncha specimens, classified into 45 species were tested using a molecular diagnosis, based on PCR amplification of phytoplasma rDNA ; less than ten species carried a pathogen, either a stolbur type or a clover phylloxy type (see Table). We obtained transmission of the stolbur type phytoplasma with the cixiid *Hyalesthes obsoletus* to grapevine, periwinkle and bindweed. It is likely that other hoppers are natural vectors of the pathogen.

	N°. positive insects carrying stolbur/ N° tested - percentage	N°. positive insects carrying clover phylloxy/ N° tested - percentage
FULGOROMORPHA		
Cixiidae :		
<i>Hyalesthes obsoletus</i> Sign.	71/288 - 25 %	0/288
CICADOMORPHA		
Cicadellidae		
<i>Mocystia crocea</i> H-S.	1/30 - 3%	0/30
<i>Euscelis lineolatus</i> Brullé	4/220 - 2%	2/220 - 1 %
<i>Rhopalopyx elongatus</i> Wagner	0/33	17/33 - 51%
<i>Adarrus multinotatus</i> Boh.	0/35	3/35

Table - species giving a positive response by PCR diagnosis in 1995

15-150

DIABROTICA VIRGIFERA VIRGIFERA LE CONTE -
POTENTIAL RISK FOR EUROPE (GROWTH AND
HARMFULNESS IN YUGOSLAVIA)

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Diabrotica virgifera Le Conte-western corn
rootworm will be new dangerous pest for more than
13 000 000 ha under maize in Europe.

Since 1992, when the first damages were
registered we have observed their biology and
harmfulness.

Due to similar climate conditions in Yugoslavia
and in American Corn Belt, *D. virgifera* adapted itself
and spread, with tendention of spreading over the other
countries in the following years.

In Yugoslavia *D. virgifera* has one generation per
year. It overwinters in the diapausing eggs which could
be found from mid July till the end of June of the
following year. Larval hatching started from mid May till
the end of June. Larvae could be found till the beginning
of August. The first imagoes could be detected from the
end of June till mid October. Egg laying began from mid
July with its maximum in August.

The larval stage makes a main damages on maiza
roots, and can destroy whole plant in severe attack.

The imagoes cause the secondary important
damages on leaf, then pollen and silk and at last on ear
tips. However, in the case of greater number of imagoes
the silk can be completely chewed and the pollination
disrupted.

15-149

SUMMARIZATION OF CHINESE ENTOMOLOGICAL LITERATURE
AND AGRICULTURAL CHEMICAL LITERATURE FROM ANCIENT
TIME TO 1995

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This article was written on the basis of the wor-
ks entitled "THE INDEX OF CHINESE ENTOMOLOGICAL
LITERATURE", "THE INDEX OF CHINESE AGRICULTURAL
CHEMICAL LITERATURE",compiled by the author him-
self.

Entomological Literature:There are 113224 pieces
of records on insects liteature.There are 10,315
pieces of records on insects literature before
the foundation of P.R.China, after that there are
102,909 pieces of records on insects literature.
It is 10 times than the time before the founda-
tion of P.R.China. There are 293 regularly and
irregularly published journals and newspapers in
China. There are 82 sorts before foundation of
China, after that there are 211 sorts.

Agricultural Chemical Literature: There are113017
pieces of records on agricultural chemical liter-
ature. Of which, 36,115 are publicly published
papers from the Chinese journals, newspapers and
books; 20,150 are literature from restricted pub-
lications; 16,925 are translated papers; 39,651
are translated abstracts from foreign sources;174
are literature on agricultural chemicals in China
written inforeign languages either by Chinese sc-
holars or by foreign scholars. Among the total of
113,017 literature, 10,125 items were published
before the founding of P.R.China while the rest
102,892 items were published after the founding
of P.R.China in 1949,with the latter outnumbering
the former by more than 10 times.

15-151

PECULIARITIES OF TRITROPIC INTERACTIONS ON DIFFERENT
CABBAGE GENOTYPES

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It is common knowledge that cabbage genotypes vary in terms and length of
some stages of plant development. These differences entail specific features of
interactions of cabbage with its main pest species at particular developmental
stages.

Delia brassicae and *Plutella maculipennis* are the main pests of cabbage at the
period from seeding to 11-14 leaf stage. No significant differencs have been
observed at that period in egg numbers of *D.brassicae* on different cabbage
genotypes. *P.maculipennis* has been found to prefer to oviposite on cabbage
plants damaged with *D.brassicae* and *Plasmodiophora brassicae* Wor.

At the second period of vegetation (from 11-14 leaf stage to head stage)
morphological differences of cabbage cultivars entail cultivar-specific
variations in root environmental conditions. These variations influence not
only behavior of the main pest species but the behavior of their
entomophagans as well. At that period *Delia floralis* F. and second generation
of *D.brassicae* place eggs on leafstalks of lower cover leaves and heads.
Cabbage root flies prefer therewith to oviposite on plants which were slightly
damaged previously by other injurious organisms. The extent of harmful
impact of phytophagans on the host plant has been observed to be
significantly lower on resistant cabbage cultivars as compared to susceptible
ones. Significant influence of characteristics of cabbage cultivars on the
percentage of root fly pupariums parasitized with specialist entomophagans
also has been revealed. In some cases the percentage of parasitised
pupariums was higher on resistant cultivars.

Intensification of activity of entomophagans and the use of particular
morphological and biochemical features of cabbage cultivars appear to be
effective tools for directional influence on biocenotic processes entailing
drastic decrease in pesticide application.

15-152

LIFE HISTORY, SEASONAL OCCURRENCE AND VARIETAL PREFERENCE of AZALEA LACE BUG *STEPHANITIS PYRIODES* SCOOT (HEMIPTERA: TINGIDAE)

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Longevities of male and female were 50.5 and 51.6 days. Egg period was 13.4 days, and nymphal period was 15.3 days at 25°C. Nymphs ecdysed four times. One female laid 235.6 eggs on the average into the underside of leaf along the midribs and veinlets mainly.

S. pyrioides had four generations in Chonbuk, 1994 and peaks of adult population in each generation were showed by late May, mid-July, mid-August, and mid-October. *S. pyrioides* overwintered with eggs.

Sancheol, Hwaboso, and Seokam occurred less ovipositional preference than Guongzahong and Red hope. Nymph survival rates of Hwaboso and Seokam showed lower than those of Guongzahong and Sancheol.

It is supposed that optimal control time could be early May and less oviposition preferences in Hwaboso and Seokam will probably be useful for selection of non-preference varieties.

15-154

PATTERNS OF LIFE CYCLE IN *APHIS GOSSYPPI* (HEMIPTERA:APHIDIDAE)

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A. gossypii has a characteristic polymorphism of life cycle pattern. We have investigated the life cycle patterns of 137 clones on the condition of short-day photoperiod at $16 \pm 1^\circ\text{C}$.

Percentage of holocyclic clone, anholocyclic clone and intermediate was 63.5%, 21.2% and 15.3%, respectively. Holocyclic clones have been divided into four types; a heteroecious type, an autoecious type, a GP1 type which has alate males and oviparae produced by a apterous female as well as a alate female and an androcyclic type which has only alate males produced by a apterous female. The heteroecious type has been found in 68 clones on 15 species of plant such as *Hibiscus syriacus*, *Celastrus orbiculatus*, *Cucumis melo* and *Solanum melongena*. The autoecious type has been found in one clone on *Rubia* sp.. GP1 type has been found in 15 clones on *Hibiscus syriacus*, *Fragaria grandiflora*, *Pyrus serotina*, *Chrysanthemum morifolium*, *Lilium* spp. and *Solanum melongena*. The androcyclic type has been found in 3 clones on *Cucumis sativus*, *Cucumis melo*, *Cucurbita* sp.. GP1 type clones could be distributed in whole Honshu Island of Japan. It may be possible to distinguish GP1 type from other types by their host plants.

15-153

BIOLOGICAL CYCLE AND DIAPAUSE IN *EUPHYLLURA OLIVINA* (COSTA) AND *EUPHYLLURA PHILLYREAE* FOERSTER (HOMOPTERA APHALARIDAE).
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Research was carried out from 1993 to 1995 in order to verify which species of *Euphyllura* occur in Tuscany. While the presence of *E. straminea* Loginova is excluded, *E. olivina* (Costa) infests occasionally olive-trees and *E. phillyreae* (Foerster) is spread on spontaneous and cultivated phillyreas. The biology of the two species, on which various Authors report discordant data, was studied in the outskirts of Florence, at Pescia (Pistoia) and in the pine-wood of Cecina (Leghorn). All the sampled females were dissected and the ovaries examined in order to determine their development, which was classified by 5 stages to fix the state of diapause (1st and 2nd stage), reproductive quiescence (3rd and 4th stage) and maturity (5th stage).

E. olivina can complete 3 life-cycles on olive-trees (among which one is facultative) overwintering at every stage. Oviposition takes place in January-February (1st facultative generation), in March-April and in October. The embryonic development lasts 10-15 days, the larval one 40-50 days. The adults of the three generations are present continuously during the year. The ones of the springlike generation (2nd) spend the months from July to September in reproductive diapause. The 2nd generation whose nymphs develop at expense of the inflorescences, causing the wilting by sucking the sap, is the most dangerous as it can cause some losses of the crop.

E. phillyreae is monovoltine and overwinters as a quiescent adult. Oviposition begins in January and lasts until May. Adults appear in June and, after having spent an obliged period of summerlike diapause, they remain in quiescence from September until January-March. This species, because of the emission of waxy secretion and honeydew, can cause aesthetical damages on ornamental hedges.

Both species spend the summer in reproductive diapause, which is caused by mean temperatures higher than 20°C and terminates in September - October. On the contrary, *E. olivina* females of the overwintering generation and of the first facultative one reach their post-diapause quiescence in only two weeks. The diapause termination and the passage to the phases of reproductive quiescence and maturity, is synchronized with the phenologic stage of the host plant which is suitable for oviposition: blooming and emission of buds.

15-155

A NEW SERIOUS PEST OF CITRUS IN ALGERIA

Phyllocnistis citrella STANTON (LEPIDOPTERA: GRACILLARIIDAE)

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Phyllocnistis citrella is a new serious pest of citrus orchards in ALGERIA. This leafminer appeared during the early spring of 1994 and became the most injurious pest on citrus because it was abundant enough on young leaves to inflict serious damages to the algerian citriculture.

In order to know the incidence of this phytophagous in the citrus insect pest complex, observations were recorded during all the flush periods.

A seasonal fluctuation in the leafminer population is noted.

In a second set of observations we try to record indigenous parasitoids of *P. citrella* for evaluating the effectiveness.

We study a project of integrated control in which cultural practices biological and chemical controls will be combined.

15-156

EGG LAYING HABIT OF THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: YPONOMEUTIDAE), AND DISTRIBUTION PATTERNS OF THE EGGS ON HOST PLANTS

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Egg laying habits were studied in oviposition cages. Sixteen plants of cabbage or Japanese radish were arranged in 4 rows with 4 plants per row. A pair of diamondback moth was released in the cage for a period of four days. The numbers of eggs on plants were examined every 24 hours. The observations were made on 21 cage units (cabbage:9, radish:12).

Micro-distribution of eggs on the plant: A total of 1,512 eggs were recorded from cabbage. Of these, 43.4, 38.7 and 2.6 per cent were on the cotyledon, the stems, and the leaves, respectively. A total of 1,867 eggs were recorded from radish. Of these, 29.8, 1.4, and 67.4 per cent were on the cotyledon, the stems, and the leaves, respectively.

Distribution patterns of eggs on the 16 plants: A female laid 30 - 50 eggs on 5 - 10 plants in one night. Distribution patterns of eggs always showed marked departure from the Poisson in the direction of aggregation. Degree of the aggregate tendency in egg distribution was studied using the C_A index which is greater than zero for contagious distribution. Young females laid eggs more contagiously than aged females. The values of C_A index were 0.79 in cabbage and 0.73 in radish plants. These are slightly less than 0.995 estimated in the natural rape field, *Brassica napus*. The diamondback moth tended to lay several eggs in a single oviposition, when she arrived on a host plant. The contagious distribution pattern of eggs may be produced by this egg laying habit.

15-157

A MODIFIED CONTAINER FOR MASS REARING OF STINK BUG, *GLAUCIAS SUBPUNCTATUS* WALKER (HETEROPTERA: PENTATOMIDAE).

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Rearing of stink bug, *Glucias subpunctatus*, had been currently done by placing its first instar nymphs in Petri dishes containing raw peanuts and wet cotton for food and water supply, respectively. However, under these conditions, the food supply easily got covered by mold, requiring constant renewal. Also, it was necessary to pour more water every 4 or 5 days. In order to avoid these inconveniences, a modified mass rearing container was designed. It was made up by two vinyl chloride cups (larger one: upper diam. of 130mm, depth of 100mm; smaller one: upper diam. of 100mm, depth of 50mm). The larger cup was placed above the smaller one, and they both were stuck together by glue. On the lid of the upper cup a hole (60mm in diam.) was opened and covered by a slightly depressed nylon gauze, on which the diet was placed. For connecting both cups, another central hole (8mm in diam.) was made on the stuck surfaces, and filled up with rolled filter paper (125mm long). Finally, distilled water was poured in the lower cup. Using this container, nymphs were able to feed the raw peanuts through gauze, and the filter paper roll was kept continuously, serving as water supply. Moreover, as the diet was placed outside the container, it did not mold. Thus, during whole rearing period, there was no need to renew neither food nor water supply. Rearing tests were started with first instar nymphs at densities of 28, 42, 56, 70 and 84 per container at 25 °C under 24L-0D, which emerged 21, 19, 18, 33 and 34 adults (i.e. 76, 46, 33, 47 and 40%), respectively. There was no prolongation on the developmental period of nymphs and no abnormally small adult has emerged in any of containers. This container may also prove efficient on rearing those other pentatomid species which are able to rear on raw peanuts.

15-158

DISTRIBUTION OF TWO EPILACHNA LADYBIRD BEETLES (COLEOPTERA: COCCINELIDAE) IN SOUTHWEST AREA OF KANAGAWA PREFECTURE, JAPAN

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Epilachna vigintioctopunctata (henceforth: Evp) and the so-called "Western Tokyo form" of *E. vigintioctomaculata*-complex (WTF)* are pests of solanaceous crops, *Solanum tuberosum* (potato), *S. melongena* (eggplant), *Lycopersicon esculentum* (tomato), etc. There, however, are differences in habitats of the two species.

The geographic distribution of some *Epilachna* ladybird beetles is in the order of *E. vigintioctomaculata*, WTF and Evp from north to south in central Japan.

Distribution of Evp and WTF was investigated at 73 points in southwest Kanagawa prefecture, which is located 60-90km southwest of Tokyo.

Evp were observed at 54 points in the southern part of the survey region. *Epilachna* ladybird beetles were generally observed in slightly and shady wet environs of coppices or the edges of hills.

Both species were observed in 14 of the same points, where the regions of two habitats overlap.

There was a wide area in the central part of the Ashigara plain, where neither species has been discovered.

The southern boundary where WTF live is determined by the mean monthly air-temperature and the mean monthly minimum air-temperature at certain points during the winter season from November to February.

The northern boundary is not fine, but it is thought to be determined by land form, microclimate and the mean monthly maximum air-temperature in the summer season.

* Looking on WTF as having evolved from *E. yasutomii* feeding on *Caulophyllum robustum* Maxim. (blue cohosh), and thus a pest insect.

15-159

THE MOST FREQUENT SPECIES AND IMPORTANCE OF THE SENN PESTS OF THE GENUS *EURYGASTER* Lap. IN YUGOSLAVIA

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The Senn pests or Wheat bugs (Heteroptera: Pentatomidae) are widely spread insects in all cereal-growing areas of Yugoslavia. However, they are most numerous in the northeastern parts of the country, especially in Vojvodina Province. The most representative species are *Eurygaster austriaca* Schrk. and *E. maura* L. with about 70% and 29% of the population, respectively. The other species, as *E. integriceps* Put. and *E. testudinaria* Geoffr. are less frequent. Species *E. integriceps* was found only in eastern parts of the country, close to the Bulgarian border, but not in Vojvodina Province where the populations of other species were most numerous.

Beginning with 1964, when the highest population of those insects was registered in Yugoslavia, the number of adults per square meter was checked in overwintering sites. The obtained data were useful to forecast the frequency of the pests in the next year. The bugs occurred in large numbers in 1964, 1968, 1969, and 1970. They had to be sprayed on large area in those years. More recently, their larger-scale occurrences were recorded in 1973, 1988, and particularly in 1993 and 1994, but without the necessity of controlling them.

15-160

OBSERVATIONS ON INSECTS VISITING FLOWERS OF CHERIMOYA AND ATEMOYA IN SICILY

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As an inadequate pollination is one of the factors limiting commercial production of *Annona* spp. in many countries, observations were carried out in Sicily at two different locations, Campobello di Mazara (Trapani) and Sciacca (Agrigento), where both Cherimoya (*Annona cherimola* Mill.) and Atemoya (*A. cherimola* X *A. squamosa*) were present. All the insects visiting flowers were surveyed and most of them identified. 48.9% (female flowers) and 36.6% (male flowers) were visited by insects. The staphylinid *Paraphloeostiba gayndahensis* (35.3%), the nitidulid *Carpophilus tersus* (32.9%), a species of Thysanoptera (22.8%) and the anthocorid *Orius laevigatus* (5.1%) were the most abundant species collected in Campobello, and *Orius laevigatus* (70.5%), a species of Thysanoptera (18.8%), a species of Heteroptera (5.5%) and *Paraphloeostiba gayndahensis* (2.4%) were the most abundant species collected in Sciacca.

15-161

A STUDY ON THE FAUNA OF CITRUS GROVES OF SOUTHERN ITALY.

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Within a research programme sponsored by the "Istituto di Agricoltura" of Acireale on the study of Arthropods of citrus groves; specimens living on the soil and tree foliage were sampled during the past years from several sites in Sicily and Calabria.

This report includes the data of the specimens caught in four sites by means of pitfall traps as well as the quantitative data on the more abundant orders of Insects and the references to the works that have already been published on this sample.

The most represented orders are Coleoptera (60%) Diptera (20%) and Hymenoptera (10%), for a total of about 60.000 specimens.

A further division of the Coleoptera has shown that the Carabidae is the most represented family (59% of the specimens). However Staphylinidae (21%) and Nitidulidae (16%) are also relatively abundant. From the study of more than 22.000 Carabidae it is possible to identify 28 species of which only 4 were found in all the sites.

The heterogeneity of the coenosis was calculated by the method of Sørensen and Renkonen and resulted statistically significant. The corological data are in agreement with similar studies on the ecology of citrus groves of Northern Italy that indicate the prevalence of widely distributed species.

However, in the case examined in this study it is worth mentioning the presence of three species of the Mediterranean area and one species endemic for Italy.

15-162

Investigation of Tobacco

Insects in Central Section of Yunnan Province

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Central Section of Yunnan Province is one of principal tobacco producing Regions. For to prevent and control of tobacco pest, requested by Yuxi Cigarette Branch Company, Yunnan Insectology Society organized twenty experts to investigate the tobacco insects of Yuxi, Jiangchuan, Tonghai, Huaining, Chengjiang, Yimen and Xingping Counties. This investigation was carried by two steps, one was for economy loss from pest and another for insects investigation. After investigation, they carried out Specimens Classification and identification in line with the standards of the Institute of Zoology of CAS (Chinese Academy of Science).

They went through more than 60 villages and 30 communities which the distance was 4000 kilometers. They were across area of 17000 hectares and collected more 7300 specimens, which included 4500 specimens inserted needle and 2800 specimens immersed. After being identified, these specimens include Arthropoda and Arachnoidea of Insecta; Gastropoda and Lamellibrachia of Mollusca; Polychaeta, Oligochaeta and Hirudinea of Annelida. They are altogether more than 100 families, 19 Orders and 7 Classes of 3 Phyla.

This activity make a thorough investigation of principal sorts of tobacco pest in Central Section of Yunnan Province, and some sorts haven't been reported in the past time. In order to check up on the distributed sorts of tobacco pest in Central Section of Yunnan Province, the investigation of the infant insects and the area not investigated will be carry out next year. Some difficult and complicated specimens are being identified, nowadays.

This investigation provided scientific basis for prevention and control of tobacco pest.

15-163

PHYTOPHAGOUS AND BENEFICIAL ARTHROPODS FOUND IN WILD GRAPEVINE, *Vitis vinifera silvestris* (Gmelin) Hegi, POPULATIONS OF THE IBERIAN PENINSULA.

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Nowadays, the wild grapevine, *Vitis vinifera silvestris* (Gmelin) Hegi, is considered a very important phylogenetic resource available for plant breeding of cultivated varieties. In spite of its significance, there is not too much information about its pests. Due to this fact, a research program was initiated in 1990 in order to obtain a check list of phytophagous and beneficial arthropods found in several populations of that dioecious subspecies of *Vitis* growing all over the Iberian Peninsula. Some of the main species cited in the report are: *Colomerus vitis* (Pagenstecher) (Acari, Eriophyidae), *Tetranychus urticae* Koch (Acari, Tetranychidae), *Typhlodromus rhenanoides* Athias-Henriot (Acari, Phytoseiidae), *Jacobyasca lybica* (De Berg) (Homoptera, Jassidae), *Bemisia tabaci* Gennadius (Homoptera, Aleyrodidae), *Arthrocnodax vitis* Rübsaamen (Diptera, Cecidomyiidae) y *Chrysoperla carnea* (Stephens) (Neuroptera, Chrysopidae).

Some considerations about high levels of resistance detected against pests and the absence of symptoms caused by phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Homoptera, Phylloxeridae) are included.

15-164

DISTRIBUTION OF *DIABROTICA VIRGIFERA VIRGIFERA* LE CONTE (COLEOPTERA: CHRYSOMELIDAE) IN YUGOSLAVIAR. Sekulić¹, T. Kerešić¹, D. Čamprag¹, I. Sivčev², B. Manojlović²¹ Faculty of Agriculture, Institute for Plant Protection, Novi Sad, Yugoslavia, - ² Institute for Plant Protection & Environment, Belgrade, Yugoslavia

Diabrotica virgifera virgifera Le Conte -Western Corn Rootworm (WCR), with other *Diabrotica* species is major problem on corn in monoculture in the USA. Estimate costs of rootworm damage and control measures range up to \$1 billion annually.

In July 1992., for the first time in Europe, damage from WCR larvae was registered in Yugoslavia, near-by Belgrade International airport. It means that pest might had been transported from America by airplane, probably in 1989-1990.

Main damages cause larvae, by feeding on roots and reducing the young corn plants' ability to take up nutrients and moisture so that plants become weakened, host to diseases, and ultimately fall over ("lodging"). In Yugoslavia, medium intensity to severe damages were recorded on 0.5, 6, 60 and 75 hectares from 1992-1995 respectively. In 1995, the frequent precipitation provided more intensive regeneration of damaged root.

The adult of WCR feeds on silks and blossoms. The result of uncontrolled beetle feeding is poor pollination, incomplete ear-fill, blank kernels, open target to diseases and finally reduced yield and quality. Searching for food, adults (mostly females) migrate by flying (supported by winds) to new maize fields and occupy new areas. In this way, during 1993 and 1994, *D.v.virgifera* expanded its range of distribution in Yugoslavia up to 40-50 km (20-25 per year). In 1995 the pest was found on 20 new localities, spreading in different directions, mostly to the North.

During the summer 1995, this corn pest, new for the Europe, already had been registered in south Hungary and east Croatia too (about 160 km north and 120 km west of Belgrade).

15-165

SPATIAL DISTRIBUTION OF THE CURCULIONIDAE COMPLEX ON CULTIVATED AMARANTHS IN SOUTH AFRICA.

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Cultivation of vegetable amaranth, especially on small scale, is a common practice in Africa. In South Africa, however the cultivation of amaranth is still in its infancy. Although wild growing amaranths are widely utilized by the rural population as food source. Of the insect pest complex occurring on amaranths, the weevils (Curculionidae) constitutes the single largest group, comprising of eight species belonging to two subfamilies (Curculioninae and Ceutorhynchinae). The different species occur on different parts of the plant probably occupying different niches on the same plant. Overlaps in the spatial distribution of some of the species occur. Broadly speaking the plant can be divided into three parts. The top third is infested by six species, the middle third by two species and the bottom third by only one species. Two species viz. *Neocleonus sannio* (Herbst) and *Gasteroclisus cuneiformis* (Fahraeus) were found over the whole plant and probably were opportunistic leaf feeders. The most injurious weevils were *Hypolixus haerens* (Boheman), mostly occupying the bottom third and a *Baris* species-complex occupying the top third with overlaps occurring in the middle part of the plant.

15-166

SPATIAL DISTRIBUTION OF LARVAE AND DAMAGE OF THE OBLIQUE BANDED LEAFROLLER IN APPLE ORCHARDS

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The oblique-banded leafroller, *Choristoneura rosaceana* Harris (Tortricidae) has become resistant to organophosphorous insecticides in late '80s. Overwintered larvae resume feeding on shoots and foliage and summer generations feed on foliage and fruit. Because many cultivars are grown in a mixed fashion, commercial orchards are heterogenous environments.

The objectives of this project were to assess 1) if there is difference in larval abundance among apple tree cultivars 2) if there was a spatial pattern in the distribution of larvae in orchards.

Geostatistics were applied to detect spatial patterns. While there were differences in larval abundance and damage among cultivars, there were no differences in spatial distribution patterns of larvae.

15-167

REGISTRATION OF THE FLIGHT ACTIVITY OF APHIDS IN MIDDLE GERMANY DURING 1985 TO 1995 WITH A 40 FEET SUCTION TRAP

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The suction trap in Aschersleben (Sachsen-Anhalt) was taken 1985 as first suction trap of Germany in operation. It has a limit of 12,2 m and a sucking performance of 2.400 m³ air per hour. Since November 1995 it is replaced by a newer model. Geographically it is localized in an area between the EURAPHID network in the western part and the suction traps of Poland, Czechia and Hungary in the eastern part of Europe.

Till now was caught with this suction trap more than 110 000 aphids. We could determine more than 80 species from 41 genera. The majority of the caught aphids was in the family of the Aphididae (91,8 %). The remaining individuals had assigned to the families of the Drepanosiphidae (1,97 %), Lachnidae (0,47 %), Pemphigidae (3,63 %), Anoeciidae (0,87 %), Thelaxidae (0,03 %), and Adelgidae (1,23 %). The absolute number of the aphids caught every year varies naturally from year to year in dependence of the weather conditions. The consequence of long bad weather periods during the mainly flight period is low numbers of captured aphids, e. g. within the years 1987 and 1991. In the year 1992 we caught till now the highest aphid number. This high number of aphids was mainly given by the summer flight of *Metopolophium dirhodum* (WALK.).

The most frequent species in the means of 1985 to 1994 were *Rhopalosiphum padi* (L.) (32,6 %), *Brevicoryne brassicae* (L.) (22,3 %), *Metopolophium dirhodum* (12,2 %), *Hyalopterus pruni* (GEOFF.) (3,8 %), *Pemphigus* spp. ((3,3 %), the *Aphis fabae*-group (2,5 %), *Brachycaudus helichrysi* (KALT.) (2,3 %), *Sitobion avenae* (F.) (2,1 %), *Cavariella aegopodii* (SCOP.) (1,8 %), and the *Myzus persicae*-group (1,5 %). All other species represents 15,5 % of the captured aphids.

An anholocyclic overwintering of the aphids in middle Germany is only possible within mild winters. A very early flight activity in the spring of the two most essential vectors of the barley yellow dwarf virus (BYDV), *R. padi* and *S. avenae*, we could observe with the suction trap after the very mild winters of the years 1989 and 1990.

15-168

ROUTINE MONITORING OF INSECT FLIGHT AT HIGHER ALTITUDES OVER FARMLAND.

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A significant proportion of the insect fauna migrating over agricultural land does so at high altitude (above 100 metres). This overflying population is a potential source of insect immigrants into the farmland, and it therefore appears to be worthy of monitoring. In this poster we describe how routine monitoring of high-flying insects has become an economic and practicable possibility with the advent of automatically-operating, vertical-looking radar^{1,2}.

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15-170

THE EFFECTS OF PESTICIDES ON NON-PEST INSECTS IN FARMLAND

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Pesticides may cause side-effects on non-pest insects. These effects have been relatively well-researched in short-term studies in the United Kingdom and elsewhere. To investigate effects of pesticide use at larger spatial and longer temporal scales, the UK Government (MAFF) initiated the "Boxworth" (1981-1991) and "SCARAB" (1990-1997) projects in England. This was to study the consequences of pesticide applications under more agriculturally and ecologically realistic conditions. This paper presents trends and fluctuations in arthropod numbers under contrasting pesticide regimes in the SCARAB project fields, which indicate that there is no irreversible long-term adverse effect of current pesticide use on commonly monitored insect species (i.e. Carabidae and Staphylinidae). However, examination of the timing of applications in relation to the time taken for recovery of populations indicates that repeating applications of broad-spectrum insecticides in consecutive seasons might lead to more protracted adverse effects (as occurred in the Boxworth project). In addition, there is evidence from the SCARAB project that less commonly monitored insects are more sensitive to pesticides than routinely monitored species in western Europe. Therefore, this paper also examines the relevance of current approaches in investigating the impact of pesticides on non-target insects in the arable farmland in relation to current changes in agricultural practices in western Europe.

15-169

FLIGHT MUSCLE DIMORPHISM OF ANOMALA SCHOENFELDTI OHAUS (COLEOPTERA: SCARABAEIDAE)

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In some scarab beetles, it is known that there are two kinds of adult females in field populations, one of which have normal flight muscles like adult males as well as other type without flight muscles. Physiological and ecological significance of such flight muscle dimorphism are not well known yet. To clarify them, we compared some characters such as the number of eggs in their ovaries, the influence of larval population densities and the flight habit between the adult females with flight muscles and without flight muscles. However, we could not find any obvious differences between them. Now we are seeking after the differences of other characters of them such as the size of their eggs and the adult life spans.

15-171

INSECTS IN FARMLAND: BIODIVERSITY AND BIOINDICATORS IN MONITORING TO DETERMINE ENVIRONMENTAL IMPACTS OF AGRICULTURAL CHANGE

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It has been estimated that over 25 thousand species of terrestrial arthropod occur in arable land in the United Kingdom. Suction sampling alone (which is specific to epigeal species) revealed the presence of over 500 species of insects in cereal fields in southern England and long-term monitoring has shown that populations of some, including those of at least 65 species of Coleoptera, have declined significantly in abundance over the last two decades. These population changes have implications not only for trophic interactions (for example, populations of insect - feeding game birds have also declined significantly) but raise important questions about which species should be monitored for detecting effects of agricultural change, given that a relatively small proportion of all those present are ever encountered in any one study. This paper utilises empirical data from ongoing large-scale insect monitoring in UK farming systems to consider the pros and cons of selecting indicator species and monitoring insect biodiversity as two approaches for assessing environmental impacts of agricultural change. Pesticide usage is used here as an example of changing agricultural practice to address the question of whether routinely monitored indicator taxa such as Carabidae and Staphylinidae are the most appropriate insects for environmental impact assessments, or whether using other bioindicator taxa could be worthwhile.

15-172

EFFECT OF DAMAGE CAUSED BY CEREAL FLIES ON DISTRIBUTION OF *APHID SITOBION AVENAE* AMONG WHEAT PLANTS AND COMBINED INJURIOUS EFFECT OF THE PESTS.E.A.Sinelnikov¹ & A.P.Golovchenko²All-Russian Institut for Plant Protection, St.-Petersburg, Russia - 1
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The investigation has been carried out in Kinel (central Russia) under field trial conditions. Spring wheat plants (cv.Kinelskaya 59) with main stems damaged by fly larvae (*Phorbia securis* Tiensuu and *Oscinella pusilla* Meig. in the ratio of about 1:1) were labelled at early tillering stage. Undamaged plants (both adjacent to the damaged ones and standing apart of them) were labelled at the same time. Colonization of test plants by aphid *Sitobion avenae* F. and fly *Chlorops pumilionis* Bjerk was determined as well as grain yield per plant.

Only 37.5 % of the labelled damaged plants survived by the end of vegetation period. 85.4% of them were colonized by *S.avenae*. By this reason it was quite impossible to reveal an independent impact of the fly larvae on yield. Aphid colonization of initially intact wheat plants constituted 20.8% - 25.0%; no significant influence of vicinity to damaged ones was noted. Almost all wheat plants damaged by *Ch.pumilionis* at the heading stage also were colonized by the aphids. The share of wheat plants colonized by both these pest species constituted 8.0% in comparison with 2.2% which could be expected in the case of independent distribution. The grain yield was decreased by 90.8% for survived plants damaged by *Ph.securis/O.pusilla* and *S.avenae*; 36.9% for plants colonized only by the aphid and 56.6% for combination of *S.avenae* with *Ch.pumilionis*. Compensation of yield losses at the cost of plants adjacent to damaged ones has not been confirmed. It can be concluded that the fly larvae improve the suitability of wheat as host plant for the aphid. Their combined impact on yield have to be taken into consideration.

15-174

NEZARA VIRIDULA AND OTHER SAP-SUCKING INSECTS AND THEIR NATURAL ENEMIES ON CASTOR IN ITALY

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Castor, *Ricinus communis* (Euphorbiaceae), has been introduced into some European Countries to produce oil for industrial purposes. In 1995 field observations were started on sap-sucking insects, mainly belonging to the families Cicadellidae (Homoptera), Miridae and Pentatomidae (Heteroptera), which attack this crop in Central Italy and other Mediterranean Countries.

Injuries to castor, caused by the cicadellids *Empoasca* spp. (leaf chlorosis and necrosis), the mirids *Lygus* spp. (apical meristem devitalization, leaf tattering) and the pentatomid *N. viridula* (flower and capsule shedding, seed piercing), were observed in several experimental fields, but percentages of damaged plants were generally low.

However, *N. viridula* seems to be a serious threat to the castor crop. Potential damage by this pentatomid was assessed in relation to crop stage and insect density by encaging the primary raceme of 2 castor varieties, with spined and spineless capsules respectively. Total shedding significantly increased (up to 100%) when *N. viridula* was introduced at flowering or at beginning of capsule development, while it was similar to the control (20-45%) when bugs were introduced at later stages of capsule development. As a consequence of shedding, the number of capsules and the seed weight per raceme decreased, although compensation occurred in one case, thus resulting in the production of heavier seeds. Feeding punctures by *N. viridula* were observed on seeds of both varieties, but percentages of pierced seeds were lower in spined capsules.

Natural control was exerted by several predators, adult parasitoids, and egg parasitoids. The most common and effective species were *Trissolcus basalis* (Scelionidae), reared from eggs of *N. viridula*, and *Anagrus atomus* (Mymaridae), from *Empoasca* spp.

15-173

TOBACCO FLEA BEETLE, *Epithrix hirtipennis* Melsh. (Coleoptera;Chrysomelidae), A NEW TOBACCO PEST IN TÜRKİYE F.TURANLI, Ş.KISMALI

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The tobacco plants are attacked by so many pests in Türkiye. *Epithrix hirtipennis* was detected for the first time in tobacco growing areas of İzmir in 1993. In this year, an outbreak of this insect was observed. Due to the severe damage, some tobacco growers have left the damaged tobacco plants in the field. The distribution, host plants and population density of tobacco flea beetle were investigated in 1994-1995. It is a widespread species which occurs in all over the tobacco growing areas of İzmir. The adults feed on epidermis of leaves and make many holes. This damage decreases both quality and quantity of leaves. The adults and the holes on the leaves were regularly counted. It has been found that maximum 340 holes on a normal sized leaf and maximum 50 adult/per plant that has 9-10 leaves. It is observed that the bottom leaves more damaged than the upper ones.

15-175

HAZEL-NUTS "CIMICIATO" ANALYSIS AND POPULATION DYNAMICS OF THE HETEROPTERA AND RHYNCHOTA FAUNA IN HAZEL-GROVES IN THE VITERBO AREA (LAZIO, ITALY); RESULTS OF THE 1993-94 RESEARCH PERIOD.

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In this paper results are reported of a two-year research conducted to assess the "cimiciato" damage entity by some *Rhynchota Heteroptera* in hazel-groves of the Viterbo area.

Studies on the population dynamics showed that the following damaging species are present: *Gonocerus acuteangulatus* (Goeze), *Nezara viridula* L., *Piezodorus lituratus* (F.), *Palomena prasina* L. and *Raphigaster nebulosa* (Poda).

In both years, the most frequent species was *G. acuteangulatus*; in particular, in 1993 captures for this species accounted by 60.3% of the whole of damaging *Rhynchota Heteroptera* captured; in 1994, this percentage was of 85.9%.

Damage evaluation consisted of a visual and organoleptic examen of nuts, which showed that really damaged nuts were very few, maximum 2.4 % of total in 1993.

Our results lead to suggest that the frequency of chemical treatments, which are systematically applied in the area, could be consistently reduced.

15-176

SIGNIFICANCE OF THE CEREAL APHIDS SALIVA

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There was conclusively confirmed that both grain aphid, *Sitobion avenae* (Fabr.) and bird cherry-oat aphid *Rhopalosiphum padi* (L.) belong to serious cereal pests. Therefore multiple aspects of the natural aphid-host plant interactions were considered. In general the present study concerns the aphids salivary enzymes in the chemical interactions. The following aspects have been investigated: 1) presence of some enzymes in the grain aphid and bird cherry-oat aphid salivary secretions; 2) sequence of secretion of the gelling (stylet sheath) and watery saliva in plant tissues; 3) path of aphid stylet across plant tissues; 4) damages to the host-plants (tracks) caused by the aphids penetrations. The saliva of the studied aphids contains both middle lamella and cell walls - breakdowning enzymes (pectin hydrolases) and digesting enzymes (carbohydrases and proteinases). There were no qualitative differences between the enzymes of the studied aphids whereas the quantitative differences were recorded. *S. avenae* showed higher activity of α - amylase trypsin, carboxypeptidase A and leucine aminopeptidase; on the contrary *R. padi* had higher activity of polygalacturonase, pectinesterase, α -, β - glucosidase and invertase. The gelling saliva was secreted at first while the path of the stylets through the epidermis and intermediate tissues, instead the watery saliva was injected into sieve elements. *S. avenae* caused irregular local damages (interruption of cell walls and whole protoplasts) in the epidermis and in the parenchyma particularly; while *R. padi* hydrolysed also the thick cell walls of sclerenchyma in order to reach phloem. The prescnee of peroxidase and polyphenol oxidase in the saliva of the aphids proves their ability to defend themselves against wide range of cereal phenolies.

15-178

EFFECTS OF DUNG BURIAL ACTIVITY OF
(*DIGITONTHIOPHAGUS* *GAZELLA* (F.) (COLEOPTERA:
SCARABAEIDAE) ON NUTRIENT CYCLE

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To assess transportation of total carbon (C) and total nitrogen (N) from cow dung to the soil by the dung beetle, we introduced the beetles into containers with fresh cow dung on soil having a depth of 20cm. After the four weeks, we measured contents of C and N in residual dung, the soil (0-2cm and 18-20cm in depth) and dung balls. The balls were buried in the lower layer of soil by dung beetles. 33-61% of C and 31-76% of N contaient in the initial dung were transported to the dung balls with huriar activity of beetles, while C and N contained the dung did not moved into lower layer without theirs activity. To search transportation of dung material to plants bodies, we introduced the beetles into containers with fresh dung marked by RbCl on the soil having a depth of 20cm and planted orchardgrass seedlings in them. After six months of planting, we measured the biomass of orchardgrass and the concentration of Rb in bodies of orchardgrass, residual dung, soil and dung balls by atomic absorption spectrophotometer. The biomass and the concentration of Rb in bodies of orchardgrass with burial activity of beetles were significantly higher than those without the burial activity. These results suggested that the dung burial activity of the beetles promoted the nutrient cycle occurring among dung, soil and orchardgrass.

15-177

DENSITY YIELD RELATIONSHIPS FOR
COLORADO POTATO BEETLE, *Leptinotarsa*
decemlineata (SAY), (COLEOPTERA,
CHRYSEMELIDAE) ADULTS AND LARVAE ON
POTATOES

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A three year study showed that the relationship between the density of Colorado potato beetle overwintering spring adults and spring larvae vis-à-vis potato yield can be described by 2 straight lines. A damage boundary of 5.8 spring adults per plant was established. There was not appreciable seasonal overlapping between adults and young larvae in the field. For summer adults, an action threshold of 10 beetles per plant is proposed. Regarding spring larvae, a damage boundary of 12 larvae per stalk was established. Depending on growing conditions, action thresholds of 4, 6 and 8 larvae per stalk are proposed.

15-179

THE EFFECT OF SIMULATED RAINFALL ON
PSEUDOCOCCUS CITRICULUS GREEN (HOMOPTERA:
PSEUDOCOCCIDAE)

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Pseudococcus citriculus is a pest of citrus in Japan. It does not usually cause serious problems in citrus orchard, but often occurs in greenhouses, reducing the value of the fruits by excreting honeydew, in which the sooty mold fungi grows. At the experimental orchards, the first generation of this pest occurred from late May to June, rainy season in Japan, and it was observed that the rainfall decreased this generation's survival rate. Potted satsuma mandarin trees infested with *P. citriculus* were exposed to simulated rainfalls delivered at rates of 5, 10, 20 and 40mm/h with and without wind at a rate of 2m/s for 30, 60, 90 and 120 minutes. Rainfalls with wind tended to wash out more *P. citriculus* individuals from the plants than those rainfalls without wind. Rainfalls of longer durations also tended to be more efficient on washing them out. Furthermore, first instar was more vulnerable to simulated rainfall than the other developmental stages. From these result, rainfall in the orchard consists in an important mortality factor to *P. citriculus*.

15-180

RESISTANCE TO BIRD CHERRY-OAT APHID AND CEREAL LEAF BEETLE IN WINTER WHEAT

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Resistance tests based on infestation by bird cherry-oat aphid (*Rhopalosiphum padi* L.) and estimation of leaf-feeding damage by cereal leaf beetle (*Oulema melanopus* L.) were conducted on 18 and 26 winter wheat varieties, respectively, in 1987-1995.

Grain yield and thousand-kernel mass were measured in infested and non-infested control plots in cages covered by insect nets. Highly significant differences were found between genotypes in infestation by bird cherry-oat aphid and feeding damage by cereal leaf beetle. Infestation by bird cherry-oat aphid varied between 20% ('GK Zombor') and 63% ('GK Lili'). The most resistant variety, 'Downy' had 20% leaf-feeding damage by cereal leaf beetle and most susceptible, 'GK Kincső' 72%. Complex effect of bird cherry-oat aphid and cereal leaf beetle was observed in 1987-1990. Yield of the most tolerant genotype ('GK Korány') was reduced by 33%, while the thousand-kernel mass was reduced only 27%. The maximum losses were 67% in yield and 54% in thousand-kernel mass. A close correlation was found between infestation severity by bird cherry-oat aphid and yield reduction ($r = 0.78$, $P < 0.001$). Principal components and multiple regression analyses were performed to determine the relationships among tested characteristics and to quantify the effect of some of them.

In 1991-1995, when cereal leaf beetle attacked alone, the most sensitive genotype, 'Mv 13' suffered 41% loss in yield, while the most resistant one, 'Downy' 18%. The correlation between feeding damage by cereal leaf beetle and yield reduction was medium ($r = 0.66$, $P < 0.001$).

Trichome length of the flag leaf exhibited a significant correlation with feeding damage by cereal leaf beetle ($r = -0.80$, $P < 0.001$).

This work was supported by OTKA grant No. T6045.

15-182

EFFECT OF LEAF PUBESCENCE IN RESISTANCE OF SOYBEAN TO THE FALSE MELON BEETLE, *ATRACHYA MENETRIESI* FALDERMANN.

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The effect of pubescence in resistance of soybean to the false melon beetle (FMB), *Atrachya menetriesi* Faldermann, was investigated in the field and laboratory using genotypes of soybean with varying levels of pubescence of leaves. There was a negative co-relationship between trichome density on leaves and levels of leaf damage caused by feeding of FMB adult. Most of the genotypes which exhibited high level of resistance to FMB were highly pubescent. There was clear difference on damage level of leaves between high pubescent variety, T-207, and glabrous variety, Mumoh-hadaka, by adult feeding in dual-choice assays. The differences of damage level of leaves between T-207 and Mumoh-hadaka did not change even after reciprocal application of methanol extracts and squeezed plant sap of both varieties on the leaf surface. It might indicate that this phenomenon does not concern with chemicals. Furthermore, the beetles attacked intensively to half parts of leaf which were removed trichomes in pubescent variety, T-207. The normal type of trichome only confirmed on soybean leaf by SEM observation. There was no glandular trichome which produce some kinds of substance. According to these results, trichomes might act as mechanical barrier to feeding behavior of the beetles. The pubescence therefore indicates to be an important factor associated with resistance of soybean to FMB.

15-181

INITIATIVES TO MANAGE THE WESTERN CORN ROOTWORM

(*CLEOPTERA:CHRYSMELIDAE*): AN OLD PEST FOR AMERICA BUT A NEW PEST FOR EUROPE

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The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is an economic pest of maize, *Zea mays*, in the United States and annually is responsible for over one billion dollars in loss and management costs. This insect is relatively new in Europe, and it is a potential pest there. Our research is focused on insect plant resistance. We have identified antibiosis in exotic maize germplasm from Mexico and South America. Other sources of resistance have been identified in germplasm of relatives of maize: *Tripsacum*, teosinte, *Zea perennis*, and *Z. diploperennis*. The level of resistance and cultivar adaptability in the various germplasms is currently being enhanced by back crossing and recurrent selection.

15-183

INTENSITY ATTACK OF EUROPEAN CORN BORER (*PYRALIDAE: OSTRINIA NUBILALIS* Hübner) ON CORN IN SLAVONIA (CROATIA)

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European corn borer (*Ostrinia nubilalis* Hübner) is a very important economic pest on corn in northerneast part of Croatia. The intensity attack is very high but its control is very poor.

In Slavonia region, appearance of European corn borer on corn was being investigated from 1985 to 1995. Every year about 50 hybrids of corn, FAO groups 100 to 700 were investigated. The dissection of plants was done during the autumn prior the harvest. Measured factors were as follows: number of holes, number of caterpillars and their position as: in ear, above the ear and below the ear, per plant.

All hybrids are attacked by this pest every year, and the highest intensity was at FAO groups 400, 500 and 600. The lowest attack was 17,05% and the highest was 98,44% (average 56,61%). The highest number of holes and caterpillars was below the ear (45,30%, 61,62%), above the ear (28,91%, 26,26%), and in the ear (25,78%, 12,12%).

Only agrotechnical measures are provided among integrated pest management. The last few years we tried to control European corn borer by biological insecticides on the base of *Bacillus thuringiensis* Berlinier. The attack has been reduced from 9 to 42%. The investigation is very important and indicates the possibilities of biological control as one measure of integrated pest management in corn production.

15-184

ENHANCING OVERWINTERING MORTALITY OF COLORADO POTATO BEETLE *LEPTINOTARSA DECEMLINEATA*

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Colorado potato beetle *Leptinotarsa decemlineata* is one of the most important pest by different kinds of fam. Solanacea. Parallel with the chemical means for insect pest control alternative plant protection solution have been proposed in our country—biopreparats, bioagents, plant extracts and different agroecological activities. The possibility for utilization the agroecological activities related to diapause ecological nature has been investigated as phenomenon by Colorado potato beetle.

The investigations have been carried out in four potato field with natural barriers from bushes, rocks and fodder agro.

In all investigated field except those of the control, potato enticing strips have been planted later and in some of them a has been used. On the basis of the so carried out investigation it has been established that the enticing strips planted with subsequent potatoes on the potato fields and fodder agro can attract the larger part of the population. The complementary food quantity makes longer the beetles' active life and it leads to discordance between the photoperiod and the related with him mechanism of the specimen endocrine system. As a result from all agricultural activities the hibernate mortality rate reach to 86% in separate fields.

15-186

NATURAL REGULATION THE INSECT-PESTS OF SPRING-RAPE IN NORD-WEST RUSSIA

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One of the question in the history of agriculture is high crops rape (*Brassica oleifera*) in Russia of the second half XIX of a century - from 16 up to 32 centners on hectare on the significant area (350 thousand of hectares). Presently 80-e years on these soils crop has lower on the order below. Partly it was called soil degradation, but one of reasons is losses of a crop from more than 100 species insect-pests. Therefore, in modern technology rape culture it is provided up to 5 - 7 insecticide treatment. Our researches have revealed two peculiarities, which enable rape cultivation without application insecticides. This promoted first of all late autumn seeding for 5-7 days before occurrence heavy snow. Due to natural seeds vernalized and plant growth in conditions extended day the crop was formed in super early terms. Thus the mass exit of main crop destroying insects occurred late formation the most favourable for their stage of development rape. In this case the term mature growth of rape appears shorter, than term of development it pests. In XX a century because of refusal from grass-arable rotation the favourable conditions for distribution crucifer weeds were created. With it dynamics of the rape pests insects is closely connected. Besides at present time the late autumn seeding is impossible, because sharp reception of long-term forecast of the occurrence steady snow cower now is practically absent.

15-185

THE ROLE OF JAPANESE BEETLES IN THE BIRD STRIKE HAZARD AT AN AIRPORT IN EASTERN UNITED STATES

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Damage related to the feeding habits of Japanese Beetles (*Popillia japonica*) is well known, yet serious problems also occur when adult beetles attract insectivorous birds to airport habitats. Many coastal airports are periodically invaded by large numbers of Laughing Gulls resulting in severe bird strike hazards that can seriously endanger aircraft and personnel. At times the problems are so severe that air operations must be curtailed resulting in significant economic and social costs. We studied the relationships between Japanese beetle populations and Laughing Gulls to develop management strategies compatible with the unique requirements of an airport environment. We worked at Atlantic City International Airport (ACY), a large (2,225 ha) urban airport serving the needs of commercial, government and private air traffic. It is situated 8 k from the Atlantic coast on the interface between an urban zone and pine-oak forests. We monitored populations of adult Japanese beetles and Laughing Gulls over four seasons. Each year the increase in gulls closely followed the increases in adult beetles. Gull numbers peaked at ACY when gulls were feeding young in colonies located 10 k away in coastal salt marshes. Diets of gulls showed heavy reliance on Japanese beetles as food for developing young in colonies. Since gulls came to ACY to obtain food for their young, we undertook management of the gull problem by controlling the adult Japanese beetles. Our management strategy was based on removal of the vegetation used as food by adult beetles (airport regulations precluded use of chemical agents). Pilot studies conducted over 3 years showed significantly lower numbers of beetles in treated areas. Determination of whether fewer beetles reduces gull numbers awaits implementation of our experimental approach over the entire airport.

15-187

ACTIVE SPACE OF A HIGH DOSE PHEROMONE POINT SOURCE MEASURED BY FIELD ELECTROANTENNOGRAM AND DISRUPTION OF TRAPPING IN AN APPLE ORCHARD

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The active space of a single pheromone point source designed for mating disruption was measured by pheromone trapping and electroantennogram recordings in an apple orchard. One hundred polyethylene dispensers were applied to a single central tree, and pheromone traps baited with high dose lures (1 mg) were placed in four radiating transects along or across rows. Catches were compared with a similar layout in an untreated block.

The prevailing northeasterly wind distorted the active space. The single point source prevented any trap catch up to 13.5 m downwind, compared to 4.5 m upwind, or to either side. The electroantennogram recordings, made at distances of 5, 10, 20, 30 and 40 m downwind indicated that pheromone bursts were sometimes detectable 40 m downwind.

This experiment suggests that deployment of dispensers may need to take distortions in the active space from a prevailing wind into account. Less distance between dispensers may be required parallel to the wind direction, compared with deployment orthogonal to the wind.

15-188

MULTI-BAITED SEX PHEROMONE TRAPS FOR *COSSUS COSSUS* AND *ZEUZERA PYRINA* MALESS. Maini, P. Riolo, R. Ferrari¹, F. Galgano¹, S. Nardi², F. Rama³

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In Italy, the two species of Lepidoptera Cossidae - European goat moth (EGM) *Cossus cossus* (L.) and Leopard moth (LM) *Zeuzera pyrina* (L.) - can infest both fruit orchards as well as other trees. EGM and LM larvae can live together on the same tree plant, burrowing galleries in trunks and branches. The chemical control is difficult and, in case of insecticide sprays, the ecological impact could be very high, especially in reforestation areas. Recently, several trials demonstrated the success of the mass trapping of EGM and LM males. Experiments were conducted to evaluate capture efficiency in relation to: 1) different types of traps (funnel with or without different kinds of baffles); 2) position of traps in the tree canopy; 3) effect of multi-baited traps *i.e.*: EGM sex pheromone dispenser added to LM dispenser in one trap compared to single-baited sex pheromone trap (standard trap). The EGM sex pheromone dispenser was a bimatrix loaded with 8 mg Z512Ac (outer rubber caps) + 4 mg Z310Ac (inner rubber septum protected by a nylon sheath) and the LM dispenser was a polyethylene cap with 9.5 mg E2Z13-18Ac + 0.5 mg E3Z13-18Ac + 0.5 mg Z2Z13-18Ac. Multifactorial analysis demonstrated that: 1) there was no difference in male captures of both species related to type of trap; 2) traps located over the tree canopy were the most efficient for EGM and LM male captures; 3) the number of males of the two Cossidae species trapped in multi-traps was not different with respect to standard traps. To our best knowledge, this is the first report showing that two moth species can be attracted simultaneously in open field at the same trap with no reduction in efficiency. Multi-baited traps are also used to monitor stored-product Phycitinae moths. However, in other Lepidoptera species, multi-baiting is usually less attractive as compared to a normal, selective and specific lure. Multi-baiting traps can be recommended either for mass trapping or survey applications for EGM and LM.

15-190

NOCTUA PRONUBA AND SPILOSOMA LUTEUM MALES ATTRACTION TO VIRGIN FEMALES AND SYNTHETIC SEX PHEROMONE OF HYPHANTRIA CUNEA

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The Fall web-worm moth (FWW) *Hyphantria cunea* Drury (Lepidoptera Arctiidae), was introduced into Italy in 1977. Studies of FWW sex pheromone trapping demonstrate the possibility of surveying its spread in our country. In 1993, '94 and '95, sticky traps baited either with synthetic sex pheromone (polyethylene cap loaded with 1 mg of: Z9, Z12, Z15 - Octadecatrienal; Z3, Z6 - 9S, 10R - Epoxyheneicosadien; 1, Z3, Z6 - 9S, 10R - Epoxyheneicosatrien) or 2-3 FWW virgin females were attractive not only to FWW males but also to the Buffer ermine (BE) *Spilosoma luteum* Hufn. (Lepidoptera Arctiidae) and the Large or European yellow underwing (EYU) *Noctua pronuba* L. (Lepidoptera Noctuidae) males. These moths are not yet included in the Am *et al.* pherolist updated to Nov. 17/1995. In unbaited traps, no males were caught. The lack of selectivity generally occurs when the bait is a blend of synthetic chemical components rather than natural bait: *i.e.* virgin females and/or sex pheromone extracted from females. This phenomenon might be explained by the fact that FWW represent an exotic species. Both BE and EYU males were caught by either natural or synthetic baits. The average number of males caught per season in each trap was about 20 for BE and EYU. *S. luteum* is a bivoltine polyphagous species harmless to crops while *N. pronuba* is a pest in several cultivated plants. The life cycle of EYU may be mono or bivoltine. In 1979, *N. pronuba* from Europe was introduced into Canada and in 1985 in USA. Therefore, a survey using traps baited with FWW sex pheromones can be helpful in detecting the spread of EYU to new areas. Further efforts are needed in order to know more about the chemical communication system of these moth species such as the cross attraction and the chemical identification of the sex pheromone components.

15-189

THE ROLE OF PHEROMONES AND AN INSECT GROWTH REGULANT IN THE MANAGEMENT OF *SYNANTHEDON TIPULIFORMIS* (CLERK) (LEPIDOPTERA: AGERIIDAE).

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A large scale, replicated field trial investigated the value of pheromones to disrupt the mating of the *Synanthedon tipuliformis* (Clerk). It involved seven blackcurrant plantations, covering 80 hectares divided into twenty two plots. It established the superior efficacy of (E,Z)-2, 13 octadecadien-1-ol acetate (known as E, Z2, 13-18:OAc), and referred to as the "Australian blend", over the binary mixture of (E,Z)-2, 13 octadecadien-1-ol acetate (known as E, Z2, 13-18:OAc) and (E,Z)-3, 13 octadecadien-1-ol acetate (known as E, Z3, 13-18:OAc), and known as the "New Zealand blend". The trial demonstrated an optimum pheromone straw density of 250 straws per hectare except where the pest population exceeded 1 larvae per metre of stem (in mid winter) and 500 straws per hectare were required. Straws were placed evenly except for concentrations at the upwind side of the plantations, assuming the prevailing north westerly winds would have greatest impact on mating disruption, but it was found the impact of sea breezes late on hot days was more important. The insect growth regulant fenoxycarb was found to be efficacious in the control of the pest and could be used to complement the impact of pheromones where the pest population was high. It also offers an alternative means of control where pheromones cannot be used and prospects for the control of other *Synanthedon* species.

15-191

FIELD OBSERVATIONS ON SEXUAL ATTRACTANT OF *PHYLLOCNISTIS CITRELLA* STANTON: (7Z,11Z)-7,11-HEXADECADIAL

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Considering the importance that pheromone traps can have in integrated control of *Phyllocnistis citrella* Stainton specific observations were carried out in the field using traps baited with 1 mg of sexual attractant (7Z,11Z)-7,11-Hexadecadienal contained in rubber capsules or in small polyethylene vials. Pheromone trap captures were compared to those of the chromotropic traps in lemon-yellow plexiglas. The pheromone baited traps captured a greater number of individuals than the chromotropic traps (14.0 adults/trap/week in traps baited with pheromone in rubber capsules and 10.7 in those baited with pheromone in plexiglas vials against 5.8 in the chromotropic traps).

The adults captured in the pheromone traps were observed under the microscope in order to determine the sex by observing the genitalia. Only 45.3% of the captured individuals were male. The attraction of both sexes to the same substance raises serious doubts as to whether it is the sexual pheromone.

15-192

IDENTIFICATION OF THE SEX PHEROMONE
OF THE LOOPER MOTH *Ctenoplosia albostrata*
(Lepidoptera: Noctuidae)

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The looper moth *Ctenoplosia albostrata* is one of the pests of chrysanthemum in Japan. To control this insect, we investigated the sex pheromone. Larvae were collected from October to December in Tsukuba and reared on leaves and an artificial diet for successive generations under 16L-8D. To isolate and identify the female sex pheromone, their abdominal tips were collected at different times of day and extracted with hexane for 30 min. The extracts were analyzed by GC-MS and GC-EAD. Then it is shown that the amount of the pheromone had a maximum peak at 3 hours after light-off and the main component is (Z)-7-dodecenyl acetate which is known as a common sex pheromone component in PLUSIINAE.

15-194

APPLICATION OF MATING DISRUPTION TECHNIQUE TO CONTROL THE
GRAPE MOTH *LOBESIA BOTRANA* (DEN. AND SCHIFF.) IN TUSCANY

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Between 1991 and 1994 the mating disruption technique (MDT) was applied to control *Lobesia botrana* (Den. and Schiff.) (Tortricidae) on a typical Sangiovese cv vineyard in the Chianti region (Tuscany). A 4 ha area was treated in 1991, which was extended to 8 in 1992, to 10 in 1993 and to 12 in 1994. Double-bulb shaped BASF dispensers containing 495 mg (1991), 240 mg (1992), 220 mg (1993) and 240 mg (1994) of the main component of the sexual pheromone of *L. botrana*, E7,Z9-12:Ac, were distributed in the vineyard at a density of 500-600/ha, before the beginning of the second flight to control both carpophagous generations. In 1991 and 1992 chemical insecticide treatments were applied to limit the first generation in order to reduce the density of the adult population.

Effectiveness of the MDT was evaluated by comparing the number of male captures per pheromone trap, the number of larvae per bunch and the number of damaged grapes per bunch recorded in four situations: the central areas of the pheromone treated vineyard (CPTV), the border areas of the pheromone treated vineyard (BPTV), the insecticide treated vineyard (ITV) and the untreated vineyard (UV).

In the pheromone treated vineyard the male captures were always highly limited; however, these were totally absent during the second and third flight only in CPTV in 1992 and 1993. In each of the four years in CPTV the mean number of larvae per bunch was 0.06, 0.17, 0.05, 0.17 for the second generation and 0.52, 0.24, 0.00, 0.69 for the third. In UV the corresponding values were 2.40, 1.37, 0.23, 0.66 for the second generation and 5.48, 3.12, 0.04, 3.18 for the third. From statistical analysis larval infestation in CPTV was always significantly lower than in UV except for the third generation in 1993 when the population density of *L. botrana* reached extremely low levels everywhere. The percentage effectiveness of MDT varied between 74.2% and 97.5%.

In 1991, 1992 and 1993 larval infestation in CPTV was statistically not different from or inferior to the one in ITV. The same result was obtained in 1994 second generation, while in the third the mean number of larvae per bunch was much higher in CPTV (0.69 vs. 0.24). Larval infestation in BPTV was always and in some cases significantly higher than in CPTV.

On the whole the MDT showed positive results when considering percentage effectiveness in comparison with the untreated control. However, this technique was not able to limit larval infestation sufficiently, particularly in the case of the third generation; consequently it is not yet suitable for vineyards in the Chianti region.

15-193

EUROPEAN CORN BORER MONITORING IN SWEET CORN:
SEX PHEROMONE AND PHENYLACETALDEHYDE MULTI-
BAITED TRAPS

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Ostrinia nubilalis (Hb.) (Lepidoptera Pyralidae) the European corn borer (ECB) can be monitored using different sampling techniques: sweeping net, egg masses scouting, light and sex pheromone traps, etc. However, some of these samplings are either time-consuming or not really related to the subsequent larval damage. Intraspecific pheromonal variability in ECB may produce low correlation between the number of males caught and corn infestation. In protected sweet pepper, the possibility of monitoring ECB infestation has already been investigated using cone traps baited with sex pheromone and Phenylacetaldehyde (PAA) (multi-baited traps). PAA is a semiochemical that attracts ECB males and females. In trials carried out in sweet pepper, the average number of females caught showed a significant correlation with fruit damage. In sweet corn, trapping experiments were conducted over a two year period with two kinds of cone traps (XLa, XLb) set up at the borders of corn fields. ECB attacks of first and second generation larvae were evaluated in the corn ears using a damage index (DI). The sex pheromone bait was a rubber stopper with 0.1 mg of a blend of: (97:3) E:Z-11-14Ac. In conjunction, the PAA bait (100 mg in a felt fibre dispenser) was added to the rubber stopper. A correlation was found between the number of females caught per trap and DI ($r=0.70$ for XLa and $r=0.60$ for XLb trap). On the contrary, the number of males caught per trap, either with XLa or XLb, were not correlated to DI. A linear model of multiple correlation fitted to the data of simultaneous captures of males and females showed that the coefficients were not as high as compared to the simple correlation. The correlation coefficients obtained by fitting a curvilinear response surface were higher (i.e. $r=0.76$ in case of XLb trap) thus indicating that the simultaneous counting of males and females in cone traps can be an efficient monitoring tool.

15-195

MONITORING THE LEAF ROLLER, *Phtheochroa cranaodes* (M.)
(LEPIDOPTERA: TORTRICIDAE) BY NATURAL AND SYNTHETIC
SEX PHEROMONE.

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The leaf roller, *Phtheochroa cranaodes* (Meyrick), is the most important lepidopteran pest of apple orchards in southern of Brazil. The damage occurs when the larvae feed on the fruit skin, reducing its commercial value. Larvae shelter in the leaves and fruits thus their control by most insecticides become very difficult. A monitoring system by natural sex pheromone (virgin caged females) has been used with some limitations, a problem that could be eliminated through its replacement by a synthetic sex pheromone. The main component of the sex pheromone produced by *P. cranaodes* female was isolated and identified as dodecadienyl acetate, based on chemical analysis and eletroantennogram assays. Field trapping revealed that the synthetic sex pheromone was as efficient as caged virgin female and it lasted for more than 9 weeks. Therefore, the results suggest that synthetic sex pheromone can replace the caged virgin female for monitoring *P. cranaodes* population in apple orchards.

15-196

TRITERPEN, AMYRIN AND AMYRIN ACETATES IN MULBERRY TREE AS FEEDING STIMULANTS OF THE YELLOW-SPOTTED LONGICORN BEETLE, *PSACOTHEA HILARIS* (PASCOE)(CERAMBYCIDAE: COLEOPTERA)

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The yellow-spotted longicorn beetles are a serious pest of Moraceae trees including *Morus* spp. and *Ficus* spp. in Japan. Recently, an infectious *Beauveria brongniartii* is developing as a biological control agent of this pest, and some semiochemicals, feeding stimulants or attractants from their host plants are requested for enhancing infection by this fungus. *n*-Hexane extracts of mulberry bark showed strong stimulation of feeding activity in the beetles. The active extracts were further fractionated and purified with silicagel CC, TLC and HPLC. By GC-MS, ¹³C-NMR analyses, four triterpens, α -amyrin, β -amyrin, α -amyrin acetate and β -amyrin acetate were identified as main components of active fractions. The fractions including these triterpens also served as a swallowing factor in feeding. Ursonic acid and oleanolic acid also induced significant feeding responses of the beetles.

15-198

Difference in host preference of *Epilachna admirabilis* (Coleoptera: Coccinellidae) among certain Cucurbitaceae

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Epilachna admirabilis Crotch is a phytophagous lady beetle which feeds on cucurbitaceous plants such as the genera *Trichosanthes*, *Melothria* and *Gynostemma*. *Gynostemma pentaphylla*, one of the host plants, was classified as strain 1 to 3 based on collected areas and was used for feeding and rearing tests.

In feeding and rearing tests, the strain 3 was less preferred to others, and the larvae died at first instar. In this results, it was thought that strain 3 may contain antifeedant(s) to *E. admirabilis*.

Fresh leaves of *G. pentaphylla* strain 1 to 3 were extracted with MeOH and partitioned successively with CHCl₃, EtOAc, BuOH and water.

Each fraction obtained was used for leaf disk bioassay. The results of bioassay showed that CHCl₃ fractions of each strain and BuOH fraction of strain 3 had antifeedant activity. As a result, BuOH fraction of strain 3 was suggested to play an important role in antifeedant activity.

15-197

THE INSECTICIDOUS INFLUENCE OF TOMATINE ON THE GERMAL CELLS OF THE COLORADO BEETLE *LEPTINOTARSA DECEMLINEATA* SAY.

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Necrosis process caused by tomatine in the cells of the Colorado beetle is similar to that caused by chemical insecticides. Necrosis takes place in a part of the male and female germ cells during the last stages of their development. The dissolution of the oogoniums and spermatogoniums has not been observed. Regarding ovocytes, the structure of the nucleus changes at first. In the pycnotical nucleus nucleoli remain out of the compact structures of chromatin. The mass of the pycnotical nuclei often flows out the cytoplasm through a breach in the nuclear membrane. The dissolution of the cytoplasm of ovocytes begins in the external part fitting to the epithelial follicular cells. Usually the epithelial follicular cells, which embracing the ovocyte, touched by the necrotical changes remain still after the disappearing of the ovocyte's nucleus and cytoplasm.

The process of necrosis touches also male germ cells - spermatides, spermatozooids. In the follicle of spermary it has been observed that the sheaves of spermatides' and spermatozooids' nuclei flow together in a dense mass. As a result of the disintegration of the ovules the amount of the Colorado beetles' eggs diminishes.

15-199

THE MYSTERY OF METHYL EUGENOL: I. WHY METHYL EUGENOL IS SO EFFECTIVE FOR CONTROLLING FRUIT FLIES

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The Oriental fruit fly, *Bactrocera dorsalis*, was eradicated from the Ryukyu Islands, Japan, using methyl eugenol (ME) and the male annihilation technique. The fly population on each island was nearly or completely eradicated within 1 year after fiber blocks (4.5 X 4.5 cm) saturated with 10 g of poisoned ME were distributed at a rate of 2 - 4 per hectare/month. Considering the notion that eradication of a pest population from an isolated area is unlikely because pest control using chemicals such as insecticides or attractants become less efficient as the population density decreases, the success of the male annihilation technique using ME is a mystery.

Many naturally occurring plants emit some natural analog of ME and many *Bactrocera* fruit fly species are strongly attracted to these plants, indicating that there is a special biological significance for the flies. Although some authors have proposed ideas to explain the biological significance of ME, the reason why eradication of *B. dorsalis* was achieved so easily remains a mystery.

Based on the results of our outdoor screen cage experiments on the attractiveness of ME to the carambola fruit fly, *Bactrocera carambolae*, and some published data, we discuss why ME is so effective in controlling fruit fly species.

15-200

GROWTH INHIBITION IN THE MILKWEED BUG, *ONCOPELTUS FASCIATUS* DALLAS BY PLUMBAGINOIDS

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Effect of 6 closely related plumbaginoids on the growth and mortality of the last instar nymphs of the milkweed bug, *Oncopeltus fasciatus* Dallas were studied by topical treatment. Plumbagin (2-methyl, 5-hydroxy, 1,4-naphthoquinone) showed high toxicity to the day 0 last instar nymphs than at any other age at 10 µg/20 mg body weight dose. The lethal dose of plumbagin for 50 % nymphal mortality was 2.75 µg/insect for day 0 last instar nymph. Plumbagin at 2 and 4 µg/insect caused growth inhibition and delayed moulting by more than 2 days as compared to control. The higher doses of plumbagin caused more mortality of nymphs. At a discriminating dose of 4 µg of plumbaginoid/insect, plumbaginoids like menadione, 1,4-naphthoquinone and plumbagin significantly inhibited growth of the last instar nymphs over the others viz., juglone, lawsone, and lapachol, besides control. Starvation also caused significant growth inhibition. The structure-activity relationship of plumbaginoids is discussed in relation to the mode of action.

15-202

THE USE OF NEEMAZAL-T/S TO CONTROL MUSEUM PESTS, FOR EXAMPLE WITH *TINEOLA BISSELLIELLA* (HUM.) (LEPIDOPTERA: TINEIDAE)

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Earlier successful studies with neem in plant protection suggested that in museums and other cultural institutions this agent may be suitable for use against museum pests too. Because of possible damages of the color pigments from exhibits it is necessary to replace chemicals like camphor or naphthalene with harmless alternatives like neem.

For the tests with woolen pieces the same dose of NeemAzal T/S was used and applied in the weight ratio 1 : 1 onto the woolen.

At 25°C and 65% r. h. 10 larvae were placed on woolen pieces of equal size. After 3 weeks, the rest surface of the woolen was digitized by use of a scanner and was evaluated with a pixel-count program. For the first time it was possible to prove the applicability of this method for objective assessment of frass damage.

The tested concentrations of neem led to a significant less frass damage.

The 2 %-solution was able to reduce the destruction by 76 % of the tested surface.

15-201

EFFECT OF SOME PLANT WATER EXTRACTS ON *PIERIS BRASSICAE* L. (LEPIDOPTERA : PIERIDAE)

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The influence of crude water extracts of *Rheum rhaponticum* L., *Matricaria inodora* L., *Artemisia absinthum* L., *Artemisia vulgaris* L., *Tanacetum vulgare* L., *Achillea millefolium* L., *Ledum palustre* L., *Sambucus racemosa* L. in different concentrations on cabbage butterfly oviposition and larval mortality was investigated.

The extract influence depended on the concentration and larval instar. Most effective were 20% extracts. Higher (25-30%) concentrations did not have significantly more effect on caterpillars. Middle instars larvae were more susceptible to extracts than young or last instars caterpillars. The high larval mortality (80% and more) was caused by extracts revealed on the third day after treatment.

All extracts had influenced on oviposition behaviour of butterflies which laid smaller number of eggs on treated plants than untreated. The treatment cabbages with extract of *T. vulgare* totally prevented butterflies eggs laying on cabbage.

Extract of *L. palustre* effected also as growth regulator - the normal pupation was interrupted in many treated last instar caterpillars.

15-203

FEEDING PREFERENCE AND EVALUATION OF THE ANTIFEEDANT ACTIVITY OF CRUDE EXTRACTS FROM *Melia azedarach* L. (MELIACEAE) AND *Chrysanthemum coronarium* L. (ASTERACEAE) ON *Lobesia botrana* Den. & Schiff. (LEPIDOPTERA: TORTRICIDAE).

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The feeding preference of the 4th larval instar of *Lobesia botrana* Den. & Schiff. (LEPIDOPTERA: TORTRICIDAE) has been stated by using the dry weight consumption of leaves of several grapevine varieties as an index. The experience was performed for two days with leaves from Palomino Fino, Sultanina, Pedro Ximénez, Cardinal varieties and wild grapevine, *Vitis vinifera silvestris* (Gmelin) Hegi. The statistical analysis of data indicated that the average consumption of leave disks from Palomino Fino was significantly the lowest.

The same procedure was used in order to evaluate the antifeedant activity of crude extracts from berries of *Melia azedarach* L. (MELIACEAE) and leaves and flowers of *Chrysanthemum coronarium* L. (ASTERACEAE). All the tests were carried out at a concentration of 40 µg/cm² of each extract. The analysis of results showed that there was a statistical significance among samples treated with *Ch. coronarium* and the control ones.

15-204

ANTIFEEDANT, FUMIGATORY AND CONTACT TOXICITY
EFFECTS OF ESSENTIAL OILS AGAINST *EPIPHYAS*
POSTVITTANA (LEPIDOPTERA: TORTRICIDAE) IN NEW
ZEALAND

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Twenty essential oils including the oils of cinnamon, chamomile, pepper, juniper, cypress, cedarwood, clove, and eucalyptus were examined for their antifeedant and fumigatory properties against *Epiphyas postvittana* (light brown apple moth). Oils of pepper, cajuput and juniper showed significant fumigatory activity against the light brown apple moth. Oil of cinnamon and pepper had highly significant antifeedant effects. Feeding toxicity was observed with two essential oils namely thyme oil, and pepper oil. The significance of the active ingredients for the observed biological activity in the oils is discussed.

15-206

PHYSIOLOGICALLY ACTIVE PLANT COMPOUNDS AS
STRESS FACTORS FOR INSECT PESTS

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The system of plant immunity to harmful organisms is based on plant properties functioning as barriers for attack. Atreptic, morphological, growth, ontogenetic and physiological barriers are recognized according to classification by Vilkova (1972) as well as a number of induced resistance barriers.

Antibiotic impact of host plants on their insect consumers is performed through combination of both rather local and general morphophysiological and biochemical alterations in organisms of the insects. These alterations entail deterioration of the physiological state of consumers resulting in their death or in a decline in their biotic potential. Eventually the reduction of numbers of phytophagous insects is achieved. Both the basal plant compounds (carbohydrates, proteins and lipids) and secondary metabolites - xenobiotics are of decisive importance in plant antibiotic influence on insect pests.

The main types of insect physiological reactivity to plant antibiotic influence expressing in terms of specific metabolic processes have been revealed by the example of a number of pest species such as colorado potato beetle, european corn borer, cotton cutworm and some others. Metabolic disruptions entail functional alterations in individual insect organs and systems. These alterations give rise to stresses of different depth depending on an extent of antibiotic influence of a resistant plant.

15-205

INSECTICIDE EFFECTS OF PLANT'S CHAPERONES

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Chaperones enriched fractions were prepared from plant biomass of the *Viciaceae* family by a new, patented in Czech agri-biotechnology. The enhanced synthesis of chaperones in the plants were induced by temperature stress (-2°C). The enriched fractions of chaperones used for experiments contained thermally stable cold-shock proteins 25-10 kDa.

The insecticide effect of the chaperone enriched fractions has been confirmed on model insect pests: *Leptinotarsa decemlineata*, *Oulema melanopus*, *Lymantria dispar*, and *Pieris brassicae*. The greatest insecticide effect of cold-shock proteins were found against early development stage of those insect which require large amounts of food. The intake, of cold-shock protein sprayed plants by the insect larvae is reduced (antifeedants), and the weight of the larvae is lower than that of the controls. The mortality of larvae were about 50-70 % during experiment. Remaining larvae of lowest weight just were dying later. The cold shock proteins influence on insect species is selective.

The insecticide results of investigated cold-shock proteins is similar with the natural, self-defense mechanism of the plants.

15-207

BIOLOGICAL ACTIVITY OF ESSENTIAL OILS AND ITS
APPLICATIONS IN ECOLOGICAL AGRICULTURE

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It is clear today more than any time that the ecological agriculture is the unique chance for soil's survival and preservation. The conception the soil is a living organism, a living system in which all must be balanced takes a central place in it.

The aim of the present investigation is to establish the essential oil's role in the ecological agriculture's context. A number of observations is carried out about the direct toxic effect of a set of essential oils active substances on economically important diseases and enemies.

On the base of the investigations it has been fixed that some of the essential oils grown in Bulgaria can be used successfully as:

- a predecessor culture acting an important role in the density reduction and mortality *Myzodes persicae* Sulz, *Aphis fabae* Scop., *Leptinotarsa decemlineata* Say, *Phytonomus variabilis* Hrbst.
- a means of acaricide toward *Tetranychus urticae* and *Schizotetranychus viticola*.
- producing a high economical effect when used in the ecological farm's crop-rotation.

15-208

USE/RISK REDUCTION PRACTICES FOR IMIDACLOPRID IN COLORADO POTATO BEETLE (COLEOPTERA: CHRYSOMELIDAE) MANAGEMENT

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The systemic/contact insecticide, imidacloprid (Admire 240FS, Bayer Corp.), can be applied as a soil treatment in many ways - one of which is a transplant drench to control Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (CPB) in tomato and eggplant. Many growers prefer this preventative approach but it is costly and may result in pest resistance, as it has for other systemic insecticides. The labeled doses are 279 to 419 grams a.i. per hectare depending on row spacing. Field studies have shown that drenches at doses as low as 46 grams a.i. per hectare applied directly to plant bedding trays prior to transplanting afforded effective protection against post-diapause adults and first generation larvae. The optimum dose may depend on the crop, cell size of the bedding tray, and time of the drench treatment. Other advantages besides cost savings included reduced handling and exposure risks associated with delivering the insecticide via the transplant water and elimination of the 12-month plant-back restriction which creates a rotational problem for many growers. Also, adjustments of the drench dose and effective protection period so that the systemic action of imidacloprid becomes ineffective between CPB generations may create a temporal refuge for susceptible individuals to reduce the risks of pest resistance.

15-209

PLANT-DERIVED SEMIOCHEMICAL BAITS: A METHOD FOR POPULATION MONITORING AND SUPPRESSION OF INSECT PESTS

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Secondary plant compounds have previously been shown by many researchers to have pronounced effects on the behavior of various insect species. Because many of these plant compounds serve as attractants or gustatory stimulants, a reasonable assumption has been that these compounds may be useful as insect baits. Baits can be used for both monitoring insect populations and suppressing pest populations. With regard to pest suppression, a bait, which may include a phagostimulant alone or a stimulant in combination with an attractant, could allow for decreased amount of toxicant relative to many standard insecticides currently on the market. SlamTM and AdiosTM (Micro Flo Company, BASF Corporation, USA) are foliar insecticides used for controlling adult *Diabrotica* pest species. These insecticides provide a model system for demonstrating the efficacy of an insect bait for suppressing an insect population. Data indicate that SlamTM and AdiosTM, which contain a floral attractant, a feeding stimulant (cucurbitacin) and carbaryl, is highly effective in luring Diabrotic beetle pest species to treated corn, cucumbers, melons and vegetables. Upon contact with the insecticidal material on the treated plants, the insects begin feeding compulsively. Ingestion of the bait in combination with the toxicant has been shown to give up to 99% mortality when applying a use rate of only 0.56 kg/ha. Because of the reduced amount of active ingredient (up to 97% reduction relative to many registered products) and high level of species specificity, natural enemies are not adversely affected. These novel insecticides show promise for safe, effective control of Diabrotic pests by using semiochemical bait technology.

15-210

EFFICACY OF EMAMECTIN BENZOATE (MK244), AC303630 (A PYRROLE), AZADIRACTIN, AFMNPV, AND *BACILLUS THURINGIENSIS* AT CONTROLLING LEPIDOPTEROUS PESTS OF CABBAGE IN CENTRAL FLORIDA WITH OBSERVATIONS ON INSECTICIDE RESISTANCE AND SAMPLING

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Emamectin benzoate (MK244), AC303630 (a pyrrole), azadirachtin, AfMNPV (a celery looper, *Anagrapha falcifera* (Kirby), multicapsid nuclear polyhedrosis virus), and *Bacillus thuringiensis* (BT) were among insecticides evaluated since 1992 for control of lepidopterous pests of head cabbage in central Florida. The percentage of plants with the bud (or head) and next four youngest leaves infested with larvae and/or pupae was used to measure insect activity. Level of infestation and damage were determined weekly. Marketability was determined at harvest. In general, level of damage at harvest was correlated with the level of diamondback moth (DBM), *Plutella xylostella* (L.), infestation beginning about halfway through crop development. Emamectin benzoate and AC303630 were effective at controlling DBM. A rotation of emamectin benzoate and BT was less effective than emamectin benzoate alone. Results with azadirachtin were varied. AfMNPV was effective on cabbage looper, *Trichoplusia ni* (Hübner), and cabbage webworm, *Hellula rogatalis* (Hulst), but not on DBM. Differences in efficacy among the BT-products were consistent with the types of insecticidal protein toxin contained in the products and toxin activity associated with BT-resistant DBM.

15-211

EFFICACY OF SELECTED INSECTICIDES AGAINST *PHYLLONISTIS CITRELLA* (Stainton) (LEPIDOPTERA: GRACILLARIDAE), THE CITRUS LEAFMINER.

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Field applications of six insecticides belonging to different chemical groups were performed against *Phyllocnistis citrella*, the citrus leafminer (CLM) in summer 1995. Fertilization effect as a factor in enhancing CLM infestation was also studied. Comparative laboratory bioassays with different immature stages of the insect were performed using the six insecticides. The efficacy of the insecticides was determined by changes in the population of the insect throughout sampling during the summer. Results have indicated significant differences between the control and other treatments. Differences in efficacy of the tested insecticides were correlated with their mode of action.

15-212

MANAGEMENT OF *EUXOA AUXILIARIS* (GROTE) AND *AGROTIS ORTHOGONIA MORRISON* IN SOUTH DAKOTA WINTER WHEAT (LEPIDOPTERA: NOCTUIDAE).

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In the absence of United States Environmental Protection Agency registered products for protection of winter wheat, field studies were initiated in 1990 to evaluate several organophosphate and pyrethroid insecticides for control of the army cutworm, *Euxoa auxiliaris* (Grote) and pale western cutworm, *Agrotis orthogonia* Morrison infestations. Sub-soil burrowing behavior (up to 10 cm below soil surface) of these pests decreased the likelihood of direct contact with applied insecticides. Thus, residual control was important in our evaluations. Naturally-infested winter wheat fields were selected for our experiment at Murdo (1990, 1993), and Wall (1992). Artificial infestation of *A. orthogonia* was carried out in studies at the Bruce (1995).

All insecticides and respective application rates provided significant ($P < 0.05$) cutworm reductions in comparison to untreated control plots. However, no significant differences were detected between either rates or compounds with naturally-infested studies. However, microencapsulated methyl parathion (PennCap-M 2FM, Elf Atochem) provided significantly superior *A. orthogonia* control at 0.84 kg (AI)/ha to that of either 0.56 and 0.28 kg rates. Additionally, our data was instrumental in supporting the registration of chlorpyrifos (Lorsban 4E, Dow Elanco) which provided growers with an efficacious method of managing these important winter wheat pests.

15-214

EFFECTS OF TRIFLUMURON ON THE EGGS AND LARVAE OF EUROPEAN GRAPEVINE MOTH, *Lobesia botrana* DEN.-SCHIFF. (LEPIDOPTERA; TORTRICIDAE) .

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In the laboratory tests triflumuron 25 WP(IGR) at the dose of 40 g/100 l water was applied topically to the different hour-old eggs and larvae of *Lobesia botrana* to search the egg development and hatch rate and the larval abnormalities and mortality rates. The effects of this compound on the egg hatch in all groups were different from control treatment, the average effect rates were calculated to be 44.21% for 24-48 hour-old eggs that were the most sensitive group.

It is observed that the treatment against the larvae caused several morphological abnormalities both the first and second instar larvae of 0-24 or 24-48 hour-old ones. Compound was also effective on the mortality rates of larvae 24-28 hour-old larvae of both first and second instars were the most sensitive group. In the field the most sensitive group to the compound was the 24-48 hour-old eggs.

15-213

THE STRATEGY OF STRUGGLE WITH GRASS MOTH (*LOXOSTEGE STRICALIS* L.) ON THE MODERN STAGE.

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An enormous extent of outbreaks of reproduction of grass moth on the Euroasian continent dictates a close collaboration of the experts from different countries for monitoring of long-term dynamics of this species, including that of the storage of diapausing pronymphs. Since the search for the ground cocoons of grass moth is rather a complicated task we can see the solution of the problem in the carrying out yearly regional inspections of the key-roots by high-mobile teams formed of the most qualified specialists.

The experience of applying the chemical means against the adult moths, which are much more sustainable to insecticide than the caterpillar, allows a highly optimistic evaluation of the idea of preventive treatments of moth congregations in the places of a mass hatching from the pupae, where the moths stay until maturity and spreading to the reproductive sites. This measure allows to decrease essentially the probability of appearance on the crops of numerous egg layings and caterpillar hearths.

Moreover, while planning and conducting the complex of protective measures against grass moth it is important to take into account a successive nature of appearing of the caterpillar hearths in accordance with the passing of migrational waves of the pest imagines. This means that within a large region suffering from the pest there is a possibility of manoeuvring with the struggle means being in disposal.

15-215

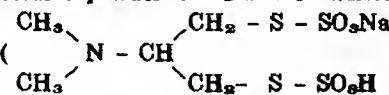
DISCUSSION ON PESTS AND DISEASE CONTROL OF RATOONING RICE IN CHONGQING

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The paper gives an introduction on investigation of pests and disease of semilate rice —ratoon rice sprouting from semilate rice stubble and research of pesticides selection from 1988 to 1994 in Chongqing, China. Damages of major pests and disease occurring in ear stage of semilate rice, such as rice plant hopper, rice borer, rice leaf roller and sheath and calm blight of rice on yields of semilate rice and ratoon rice were evaluated, and relation of yield losses between semilate rice and ratoon rice affected by these pests and disease was discussed. Analysis showed that the relativity of yield losses between semilate rice and ratoon rice was quite evident. Based on evaluation, 5~10% yield loss of semilate rice in ordinary year in Chongqing on an average was from damages of pests and disease mentioned above, and then, related to a yield loss of ratoon rice of 9~10%. In year of outbreaks of pests and disease, yield loss of semilate rice reached to 11~38%, and resulted in a yield loss of ratoon rice of 11~100%. It was concluded that effective pest and disease control in ear stage of semilate rice was important for culture of ratoon rice.

Two pesticides i.e. 50% wettable powder of Puminisi which is a complex of Shachongdan (



) and Buprofezin, and 5% granule of Shachongdan were recommended. Field tests showed they were quite effective in controlling while applied with validamycin A.

15-216

BIOLOGICAL STUDIES ON OSTRINIA FURNACALIS SPECIES GROUP OF JAPAN (LEPIDOPTERA: PYRALIDAE)

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Nine species of the genus Ostrinia have been recorded in Japan. Among them 5 species including O. furnacalis are much close to each other (furnacalis species group). The species of this group are distinguished by slight morphological differences, and the biological information is relatively limited except for O. furnacalis due to the difficulty for identification.

The biological studies, i. e., larval development, post-diapause development, host plant preference, and so on, revealed that these species were confirmed to be distinct from each other. And one species related to O. zagliaevi was newly recognized from a southern island of Japan. Some new morphological characters for identifying these species are also discussed.

Section 16

Forest Entomology

16-001

EXTERNAL THREATS: PROCESSES FOR DETERMINING THE RISKS TO BRITISH FORESTS FROM EXOTIC INSECT PESTS

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Britain's island status has a number of consequences for its flora and fauna. Following the last ice age, the number of tree species and their associated insects have been impoverished relative to mainland Europe. Forestry, as a component of the landscape and, particularly, commercially, is dominated by exotic tree species. For example, Sitka spruce (*Picea sitchensis*), Norway spruce (*Picea abies*), Corsican pine (*Pinus nigra* var. *maritima*) and lodgepole pine (*Pinus contorta*) are the main commercial conifer species.

The expansion of forestry from the early 1950s has been accompanied by an increase in the number of exotic insect pests that have established in Britain. Notable among these have been the bark beetles (Coleoptera: Scolytidae) *Ips cembrae*, *Ips sexdentatus* and *Dendroctonus micans*, the latter having been discovered only in 1982.

Recent interest has focused on the so-called Asian strain of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) which has been causing major problems in Germany and was found for the first time in Britain during 1995.

These, and other examples, will be discussed within the context of Pest Risk Analysis (PRA) with particular reference to Britain. This will concentrate on the processes of identification of potential pests in other countries, the likelihood of those pests being transported to Britain and the potential for establishment and damage should the pests get through plant health barriers. Consideration will be given to the risk factors arising from the increase in forestry in Britain and on the absence of natural enemies of most exotic potential pests, even those that are present in neighbouring countries on mainland Europe. Risk mitigation measures will then be assessed in relation to the key potential pests identified in the PRA process. These will be discussed and evaluated in relation to their success and how they might be improved to ensure future freedom from exotic pests.

16-003

FOREST PEST RISK ANALYSIS AND ESTABLISHED FOREST AND TIMBER INSECTS IN NEW ZEALAND.

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Since the early days of European colonisation New Zealand has been a trading nation and as a consequence has acquired many insect species from overseas. Approximately 170 overseas forest and timber insects are established in NZ and over 600 species of wood- and bark-boring insects have been intercepted at ports. Most of the establishments have been relatively innocuous but a few have had a major effect and some have influenced the choice of tree species in commercial plantations. No overseas insects have had an serious effect on indigenous forests.

NZ has very strict quarantine regulations that are strictly enforced; this combined with the country's remote location means that quarantine procedures are more likely to be effective than in continental countries. During the past 20 years active and passive (desk) pest risk analyses of both commodities and pest species have played a major part in influencing quarantine regulations and procedures. Examples of this, including the way New Zealand has dealt with the threats posed by pine wilt nematode (*Bursaphelenchus xylophilus*) and Asian gypsy moth (*Lymantria dispar*), are discussed.

16-002

ASSESSING THE RISK POSED BY SOME FOREST INSECT PESTS TO THE UNITED KINGDOM

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Lymantria dispar is an important Lepidopteran pest of forestry and amenity woodland in continental Europe. However, from 1907, when *L. dispar* became extinct in the United Kingdom, until June 1995, when a breeding colony was found in North London, only migrant males of this species have been found naturally in the UK. This paper explores some of the methods which can be used to assess and manage the risks posed by *L. dispar* to the UK in the light of the new outbreak.

Particular emphasis will be given to the role of climate in determining the potential for establishment of this species in the deciduous woodland of southern Britain. An egg phenology model will be employed to predict egg hatch dates at the outbreak site to assist eradication measures and development will be predicted at other key UK locations. Climatic comparisons will also be made between the UK and other areas in Europe which are subject to severe outbreaks of *L. dispar* to determine whether sufficient temperature accumulates during the summer to enable the life cycle to be completed.

The implications of these analyses will be taken into account in a review of the risks posed by *Hyphantria cunea*, another Lepidopteran tree pest in continental Europe, to UK forestry and horticulture.

16-004

THE ROLE OF CLIMEX IN PEST RISK ANALYSIS

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The volume of international trade poses escalating threats to the integrity of many natural and human-modified systems around the world by spreading exotic species of pests, diseases and weeds. Meanwhile the World Trade Organisation and the FAO have called for harmonisation of quarantine procedures. Given the great disparity between the capacities of developed and developing countries to undertake pest risk assessments, and the global value of all pest related information, there will be major benefits in the adoption of some common tools and languages to exchange information. CLIMEX is one such tool. It uses a simple file of parameter values to describe the key climatic requirements of species, without the need for detailed knowledge of their lifecycles. CLIMEX makes risk assessments, in relation to climate and hence geographical locations, transparent and so CLIMEX files can be used as an international medium for the exchange of pest risk information. At the time of writing, CLIMEX is being used in about 120 laboratories world wide, and it has been applied to over 100 species of insects, fungi and plants with equal success. It is being considered for linking with the CABI entomology database and offers great opportunities for expanding the options available for quarantine pest risk analyses, with significant new insights being gained into each new species that it is applied to.

16-006

RISK MANAGEMENT OF PINE WOOD NEMATODE
INVASION AND THE BARRIER ZONE DEFENSE
PROJECT IN JAPAN

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The pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Nematode: Aphelenchoididae), which causes pine wilt disease (PWD), is believed to be native in North America and is thought to have been introduced to Japan in logs imported in the early 1900s. By 1984 approximately 25% of pine forests in Japan were infested. Although the Japanese pine sawyer, *Monochamus alternatus* (Coleoptera: Cerambycidae), is the most important insect vector of PWN in Japan, human transportation has been more closely related to the spread of PWN. The PWN has been expanding its range by both neighborhood diffusion and long-distance dispersal. The long-distance dispersal was sometimes over 100 km, which is obviously caused not by the beetles but by humans. In northern Japan, the PWN was first found in 1975 over 200 km away from the edge of the nearest population front. Introduction is thought to have begun with the most aggressive forms of PWN transported by ship to the paper factory. Because low temperatures cause a delay in host symptom development and a slower rate of nematode population increase, the spread of the PWN and the number of infested pine trees have been slowed down in N Japan. This fact means that the control efforts and the intrinsic rate of increase of PWD are balanced in these regions. It is speculated that the PWN will expand slowly in N Japan by the repeating processes of 'spread and establishment into uninfested areas in hot dry summers' and 'keeping the status quo in cool summers'. To stop the new invasion in the hot dry summer is the key to the control strategy in the northern extremes of the nematode's range. A risk of establishing the PWN population in uninfested areas was assessed by a thermal constant above developmental threshold of *M. alternatus*. I will also introduce the barrier zone defense project in the expanding front of Japan.

16-005

CURRENT AND POSSIBLE FUTURE EXOTIC FOREST INSECTS
IN NORTH AMERICA: THE NORTH AMERICAN EXOTIC FOREST
PEST LIST PROJECT

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More than 370 exotic forest insects are now established in North America, many of which have greatly altered forest ecosystem diversity, function, and productivity. These exotics represent 10 insect orders, being mostly Homoptera (31%), Coleoptera (28%), Lepidoptera (22%), and Hymenoptera (10%). The scolytid *Tomicus piniperda* is one of the most recent exotics to become established in North America, being first detected in 1992 in Ohio. Of the exotic forest insects now in North America, 78% came from Europe, 20% from Asia, 1% from Africa, and 1% from Australia. Some of the North American tree genera that support the most exotic insect species are, in decreasing order, *Prunus*, *Pinus*, *Salix*, *Malus*, *Populus*, *Quercus*, *Betula*, *Acer*, *Picea*, *Alnus*, *Ulmus*, *Crataegus*, and *Abies*. Most of these exotic insects arrived during the past 100 years. Future introductions of new forest pests are likely due to increasing world trade, shipments of equipment and vehicles from overseas military bases back to North America, and importation of whole logs from other countries.

The North American Forestry Commission, Forest Insect & Disease Working Group has recently initiated an effort to create a list of exotic forest insects (and other pests) to exclude from North America. Most members of this working group represent federal forestry and quarantine agencies in Canada, Mexico, and the United States. It is hoped that this list will allow quarantine efforts to be focused on those pests that pose the greatest risk to North American forest ecosystems. This talk will present an overview of the project, including its goals, criteria, and progress to date.

16-007

INTRODUCTION AND SPREAD OF EXOTIC TREE SEED PESTS IN
EUROPE vs. COLONIZATION OF EXOTIC TREES BY NATIVE
EUROPEAN SEED PESTS: ARE THE PROCESSES SIMILAR ?

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Worldwide development of tree-improvement programs, especially establishment of seed orchards, has renewed attention to insects infesting tree seeds. The more as the development of international seed trading includes the potential introduction of exotic pests with seed lots, when ineffective phytosanitary measures are applied at borders. A European survey of the *Megastigmus* spp. (Hymenoptera: Torymidae) attacking tree seeds showed a total of 19 species, of which 7 were introduced to Europe. Among them, at least 3 species have been introduced from North America during the recent years. Most of these insects seemed capable of developing in unfertilized seeds as a survival adaptation compensating for pollination problems.
The potential host ranges of the introduced chalcids were surveyed and compared to those of native seed insects. The establishment and spread of exotic seed pests in the area of introduction appeared to depend on the presence of native tree species congeneric to the original host. When such species existed (e.g., firs), the exotic chalcids were observed to shift on them but the competition with native pests for seed exploitation limited their impact. By contrast, the exotic pests did not attack any native species when none of them were congeneric to the original host (e.g., Douglas-fir). In that case, the seed chalcid introduced along with the host tended to occupy the entire niche because of the absence of competitors, and parasites as well. For example, the Douglas-fir seed chalcid now occurs at high densities throughout Europe, including remote southern areas, and patterns of seed exploitation largely differ from those observed in the native areas. Introduction of seed chalcids of *Chamaecyparis*, or *Cryptomeria* from Asia to Europe is expected to result in a similar situation.
The process of colonization of exotic trees by native seed insects seemed somewhat similar. Most exotic trees introduced to Europe showed a large colonization by native insects specialized in exploiting the seed resource of congeneric native species, e.g. spruces. When no native congeneric tree species existed, colonization by native insects remained limited, and only proceeded from species poorly specialized in seed exploitation.

16-008

EPIDEMIOLOGICAL ASPECTS OF GEOGRAPHIC WITHIN - RANGE TRANSLOCATION OF *PITYOGENES CHALCOGRAPHUS* LINNÉ POPULATIONS (COLEOPTERA: SCOLYTIDAE)

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Populations of the spruce bark beetle, *Pityogenes chalcographus* L., in Europe show genetic variation, partially due to their phylogeographic position. Its biological manifestation was experimentally tested, considering parameters which could be relevant for the epidemic potential of the insects. The results indicate that *P. chalcographus* populations indeed can differ in respect of such traits, e.g. host tree specificity, speed of development etc.. Cross-breeding of beetles from distinct populations caused various effects between the parental partners as well as in the F1 progeny, depending on the origin of parents. Between partners partial incompatibility on the ethological, physiological and cytological level could be ascertained. In some F1 crossings heterosis effects in terms of vigour, fecundity and aggressiveness became visible.

It is concluded that (1) differences in the epidemic potential between distinct populations of *P. chalcographus* may be based on the phylogeographic and ecogeographic differentiation of its genetical structures; (2) the diversity of environments, where *P. chalcographus* can exist and where it is specifically adapted to in the form of local non-epidemic populations, demonstrates its ecological adaptability; (3) this implies the risk of coincidence of ecologically over-adapted alien genotypes with conditions epidemiologically most appropriate for them, when being transferred by timber trade within the geographic range of the beetle; (4) within - range translocation of beetles may also result in genetically new types of populations, in which heterosis effects raise the inclination to epidemics.

16-010

INSECT VECTORS OF PINWOOD NEMATODE: CONSEQUENCES OF INTERNATIONAL MOVEMENT OF *MONOCHAMUS* SPP.

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The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhner) Nickle, has been collected from over 20 species of *Pinus* in North America. The nematode is dependent upon adult beetles in the genus *Monochamus* (Coleoptera: Cerambycidae) for transport from infected to non-infected pines. In North America, the nematode is a common secondary pathogen of dying trees but only rarely causes pine wilt, usually on susceptible species of exotic pines. The nematode was introduced into northeastern Asia early in this century and has devastated pine forests in throughout Japan and in parts of China, Taiwan and South Korea. The nematode is not known to exist in Europe, however, suitable insect vectors do exist. Europe faces the threat, therefore, that the nematode could become established and spread if it were introduced. Such introduction could come in the form of infected wood or through the introduction of nematode infested beetles. Comparative studies of two species of vectors, *M. carolinensis* from the midwestern United States, and *M. alternatus* from northeastern Asia suggest that the reproductive and transmission efficiency does not differ greatly between the species. Adult, nematode-infested, *Monochamus* females do have the ability to transmit the nematode to new host material. This suggests that the introduction of a single, unmated adult female could introduce the nematode to new geographical locations where it could form an association with potential native vectors.

16-009

A PHYLOGEOGRAPHIC ANALYSIS OF EUROPEAN *IPS TYPOGRAPHUS* (L.) (COLEOPTERA, SCOLYTIDAE) POPULATIONSCh. Stauffer^{1,2}, F. Lakatos^{1,3}, R. Blecha¹, G.M. Hewitt²¹ Institut f. Forstentomologie, Univ. f. Bodenkultur, 1190 Vienna² School of Biological Sciences, UEA Norwich NR4 7TJ³ Department of Forest Entomology, H-9400 Sopron

Ips typographus (L.) is an important pest in the European spruce forests. The European *I. typographus* populations vary in many aspects like aggressiveness or reaction towards synthetic pheromones. These phenotypic-behavioural differences suggest that the populations are genetically distinct. There is little knowledge about the genetic variability of the populations within Europe. Population genetics can provide information on the origin of established populations, gene flow among populations and sometimes also anthropogenic influences. Here 21 European populations collected from logs were investigated by screening isozymes and sequencing three regions of the mitochondrial genome.

The northern populations had in average less alleles than the other populations and the genetic variation was lower. The northern populations as well as the populations from border zones (non-autochthonous spruce stands) had less gene flow. Although the estimation of the gene flow was high among the populations a geographic isolation with distance was detected. The sequence results (892 bp) revealed very low variation with eight mitotypes detected over all populations. The populations from Italy and Croatia were variable having most mitotypes. Most populations had only one to two mitotypes.

The geographic pattern of the geno- mitotypes might have evolved by postglacial processes of colonisation and migration. We suggest that in the last glacial periods the refugial zones were in the south (e.g. Dinaric Alps and Apennine), where most geno- mitotypes still inhabit the spruce stands. From these areas beetles re migrated to central and northern Europe. Some of the geno- mitotypes colonized successfully, some did not. This would explain the North South cline concerning the variability. Consequences of the results will be discussed: significance of the source of introduced populations for its epidemic potential; effects of wood transports; selection of geno- mitotypes might be responsible for the different reaction of the European *I. typographus* populations towards the synthetic pheromones.

16-011

A COMPARISON OF THE PEST STATUS OF AUSTRALIAN INSECTS FEEDING ON *EUCALYPTUS* SPP IN AUSTRALIA AND OTHER COUNTRIES.

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Eucalyptus spp, which are largely endemic to Australia, have been introduced to many countries and grown in plantations for hardwood production. There are a number of records of Australian insects, including *Phoracantha semipunctata*, *Gonipterus* spp. and *Paropsis charybdis* becoming pests of *Eucalyptus* in other countries yet not attaining pest status in Australia. The most obvious explanation for this phenomena is release from natural enemies in the introduced range. However, the differences in pest status and biology of the insects in exotic locations cannot be fully explained by a lack of natural enemies. Other aspects such as host range and geographic distribution in Australia, competing species and differences in silvicultural practices will be discussed.

16-012

SYSTEMATICS AND BIOLOGY OF PARASITOIDS OF EUCALYPT PESTS IN AUSTRALIA - THE PROBLEM "DOWN UNDER"

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The complex of hymenopteran and dipteran parasitoids associated with various defoliating and wood-boring insects of eucalypts in Australia can include 20 species or more. These complexes comprise an array of function types, e.g. primary and hyperparasitoids, idiobionts and koinobionts, solitary and gregarious species, mostly belonging to the Ichneumonoidea and Chalcidoidea. They provide the source of potential biocontrol agents of eucalypt pests outside Australia, but in this respect several problems are evident. Of prime importance is that serious eucalypt pests overseas are not necessarily critical pests in Australia, and therefore their biology and taxonomy is often poorly known. This was the case for the parasitoids of the cerambycid *Phoracantha semipunctata* and the pergid sawfly *Phylacteophaga froggatti*. In these cases, a large proportion of their parasitoids represented new or ill-defined species and, for *P. froggatti*, biological studies showed that two species being considered for introduction into New Zealand were facultative hyperparasitoids of the most common primary parasitoids, *Bracon* spp., and therefore potentially damaging to any biocontrol efforts.

Not only are there taxonomic problems with parasitoid complexes but also with some pest groups themselves, as shown by recent studies on *Phoracantha* and *Phylacteophaga*. Further, the rate at which taxonomic and biological knowledge is accumulating on parasitoids of eucalypt-feeding insects is low because of the paucity of research staff in Australia. Currently, there are only four parasitoid taxonomists in Australia, two of which are part-time.

Although such drawbacks exist, the chances for success of biocontrol of eucalypt pests outside of Australia is high. Eucalypt-feeding insects and their parasitoids are relatively specific and are therefore unlikely to move onto other plants and insect hosts, respectively. Further, taxonomic problems can be overcome through collaboration with overseas workers and by focusing research more narrowly. These aspects will be discussed using the above species and others as case study examples.

16-014

INSECTS AND THEIR POTENTIAL DAMAGE IN EUCALYPT PLANTATIONS OF EASTERN TAIWAN

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One light trap was set at each of two eucalypt plantations (one mainly composed of *E. grandis* and the other *E. grandis* and *E. camaldulensis*) in Eastern Taiwan. Insects were light trapped from 1830 to 2200 once or 2 consecutive nights each month. A total of 34,912 insects of 15 orders were light trapped during the period from December 1993 to November 1994. Significant differences were found among the number of insects caught monthly and among the number of trapped orders of insects. Lepidoptera were the most abundant insects, representing a about 1/3 of the total insects collected. At least 469 species belonging to 22 families could be identified from the trapped moths. No significant difference was found between the number of moth species collected from 2 plantations. Most of the important pest species damaging eucalypt plantations before 1992 (e.g., hepialid *Endoclyta sinensis*) were either not trapped or trapped with low numbers. The dynamics of trapped insects through the year is described. Implication of the results from this study to forest management is discussed.

16-013

BIOLOGY AND MANAGEMENT OF INTRODUCED ARTHROPOD PESTS OF EUCALYPTUS: SITUATION IN SOUTH AMERICA

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Most of the South American forest plantation areas are covered with *Eucalyptus* species. The largest areas are located in Brazil (3,600,000 ha), Argentina (231,000 ha), and Chile (207,000 ha). Large areas are located in Colombia and Peru, and median or minor areas in Paraguay, Bolivia, Suriname, Ecuador, French Guyana, and Guyana. Since its introduction *Eucalyptus* spp. have been attacked by a high number of native insect pests of the Orders: Lepidoptera (defoliating caterpillars), Coleoptera (borers or defoliators), Hymenoptera (leaf-cutting ants), and Isoptera (root or trunk termites). The introduced species such as the eucalypt snout beetle, *Gonipterus* spp. (Curculionidae), and the eucalypt borer, *Phoracantha semipunctata* (Cerambycidae), both native from Australia, are not really a problem at present. The overall breadth of insects on *Eucalyptus* spp. in South America and the damage caused by the most serious native pests are presented.

16-015

ECOLOGY OF THE EUCALYPTUS LONGHORNED BORER (COLEOPTERA: CERAMBYCIDAE) IN CALIFORNIA

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The eucalyptus longhorned borer, *Phoracantha semipunctata* F., was first discovered in Southern California in 1984 and continues to expand its distribution. The adult beetles are attracted to fallen eucalyptus branches, freshly cut wood, and even living trees. On these hosts the males compete aggressively for mates, and larger males have a reproductive advantage. Females deposit eggs under loose bark and the larvae mine along the cambium.

Eucalyptus species vary greatly in their resistance to borer attack, and adult *P. semipunctata* show an oviposition preference for species that are the highest quality hosts for their larvae. Resistance of living trees is strongly affected by environmental factors. In particular, moisture availability influences host resistance; turgid bark of healthy trees presents a physical barrier to colonization by borer larvae. Trees under water deficit are attractive to the adult beetles and vulnerable to attack by the larvae. Because eucalyptus in California are commonly of susceptible species and occur in poor growing conditions, the borer is a serious and economically important pest.

16-016

INTRODUCTION OF NATURAL ENEMIES FOR
BIOLOGICAL CONTROL OF THE EUCALYPTUS
LONGHORNED BORER (COLEOPTERA:
CERAMBYCIDAE) IN CALIFORNIA

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An encyrtid egg parasitoid, *Avetianella longoi*, and four braconid larval parasitoids, *Syngaster lepidus*, *Callibracon limbatus*, *Jarra phoracantha*, and *Jarra maculipennis*, have been imported into California from Australia and established in rearing colonies for mass production and release. Releases of *A. longoi* and *S. lepidus* have been conducted in coastal and inland areas and it appears that *A. longoi* has become established in several sites. This parasitoid can be reared in large numbers in laboratory colonies; female fecundity is ca. 200 eggs and development from egg to adult takes < 17 days at 25 °C. Females are very efficient at finding suitable beetle egg masses in the field with a mean parasitism rate of >90%. There are indications that *S. lepidus* has also established in at least one site but research releases of the larval parasitoids will continue to be made. Progress is slower with the larval parasitoids than with *A. longoi* because of more labor-intensive rearing procedures, longer developmental times, and more difficult field detection and evaluation methods. Once the natural enemies are established, the combined effect of improved cultural management of established trees, planting of more resistant *Eucalyptus* species, and biological control should reduce the amount of tree mortality caused by the beetle.

16-018

PESTS AND DISEASES IN PLANTATION *EUCALYPTUS*:
A GLOBAL PERSPECTIVE

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The intensive propagation of various *Eucalyptus* species as exotics in plantations is a growing trend in many parts of the world. Currently, in excess of 8 million hectares have been planted to these trees outside their native range. These plantations have thus become a major source of timber and particularly pulpwood, internationally. Numerous insect pests and diseases have resulted in serious losses to these plantings in many parts of the world. These include both pests that are known to occur on eucalypts where they are native, and others that are endemic to areas where these trees are being planted as exotics.

All indications are that losses due to pests and diseases in plantation eucalypts will continue to increase substantially in the future. Many factors will contribute to this situation which, in the longer term, will significantly complicate the propagation of these trees. New introductions of pests and pathogens are being recorded regularly. This trend will most likely continue due to increased international trade and tourism and the difficulties associated with the application of effective quarantine. Fascinating examples are also beginning to emerge, where relatively host specific pathogens of other myrtaceous genera have apparently adapted to virulence on *Eucalyptus*. These "new" pathogens not only threaten exotic plantation trees, but also *Eucalyptus* species in their native range. In this presentation, I will outline examples of these emerging trends that, in my view, not only threaten commercial forestry, but also global forest biodiversity.

16-017

CONTROL OF THE EUCALYPTUS SNOOT BEETLE
GONIPTERUS SCUTELLATUS GYLLENHAL IN
CALIFORNIA BY THE EGG PARASITOID *ANAPHES*
NITENS (GIRAULT) (COLEOPTERA:
CURCULIONIDAE - HYMENOPTERA: MYMARIDAE).
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The Eucalyptus snout beetle *Gonipterus scutellatus* Gyllenhal is a serious pest of Eucalyptus in many areas of the world. It was first detected in California in spring of 1994, and, as in other countries, it spread quickly from the original site of infestation. A biological control program was rapidly developed, using the egg parasitoid *Anaphes nitens* (Girault), with the first parasitoid releases occurring in early fall of 1994. One year later, densities of all life stages of the beetle at release sites had decreased dramatically, to the extent that beetles were difficult or impossible to find. Furthermore, defoliation levels had decreased from greater than 80% before releases to less than 10%, and are still dropping. Further work in 1996 will concentrate on releasing parasitoids at new sites of infestation. The high efficiency of the parasitoid and its rapid deployment once beetle populations were detected may prevent the beetle from becoming a serious pest in California.

16-019

POTENTIAL ROLE OF ENTOMOPATHOGENS IN THE
CONTROL OF EUCALYPT DEFOLIATORS.

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Commercial utilization of *Eucalyptus* spp. is a major component of the Australian rural economy. Historically, commercial eucalypts have been defoliated by a complex of leaf feeding beetles and moths which results in reduced growth and yields and delayed harvest.

Current control relies on the use of insecticides which, though effective, destroy natural enemies and other biota. Furthermore, aerial spraying of forests adds to public concerns about human health and the environment.

We report on the natural occurrence of entomopathogenic nematodes and fungi as components in the natural biocontrol of paropsine chrysomelids and the potential deployment of strains of local *Beauveria* and exotic *Metarrhizium* as biocides for the control of adults and larvae of a major pest, *Chrysophtharta bimaculata*.

16-020

CONTROL OPTIONS FOR THE LEAF BEETLE, *CHRYSOPHTHARTA BIMACULATA* (OLIVIER) (COLEOPTERA: CHRYSOMELIDAE) IN EUCALYPT PLANTATIONS IN TASMANIA

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The leaf beetle, *Chrysophtharta bimaculata* is a major defoliator of eucalypts in Tasmania causing significant reduction in growth rates and poor tree form. The current control strategy relies on monitoring of pest populations, predation by a suite of natural enemies and aerial spraying of synthetic pyrethroids to keep larval populations below an economic threshold. Biological, silvicultural and genetic options are being investigated in order to achieve more effective and environmentally benign control of this pest.

Microbial agents, particularly *Bacillus thuringiensis* var. *tenebrionis* (*B.t.t.*) have some potential for replacing synthetic pyrethroids in control programmes. *B.t.t.* is effective against eggs and young larvae and prescriptions for aerial spraying operations have been developed. However, *B.t.t.* has no effect on adult *C. bimaculata* which account for considerable defoliation. Investigations into the use of *Eucalyptus regnans* and *E. delegatensis* as trap trees within plantations of the more commercially favoured *E. nitens* have shown that populations of *C. bimaculata* can be concentrated on the trap species. Some provenances and families of *E. nitens* have been found to be less susceptible to defoliation and appropriate genetic selections may reduce the impact of this pest in the longer term.

The integration of these control options into an improved management strategy for *C. bimaculata* is discussed.

16-022

EXPANSION OF THE RANGE OF *LYMANTRIA DISPAR* IN NORTH AMERICA: ECOLOGY AND MANAGEMENT

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The gypsy moth was accidentally introduced near Boston in 1869 and since then has been expanding its range through much of northeastern North America. Because the gypsy moth is highly polyphagous, it is likely to become established in nearly all forested portions of North America but outbreaks will likely occur in less than half of these areas. The expansion of the gypsy moth's range can be attributed to three population processes: reproduction, short distance dispersal by 1st instars, and long-range dispersal of various life stages caused by humans. Analyses of historical data indicate that the rate of gypsy moth spread in North America has increased over the last 100 years; spread is currently occurring at about 20 km/yr. A large program designed to evaluate the feasibility of slowing the spread of this insect using a barrier zone is currently being tested. Results to date indicate that this program has been at least partially successful in slowing gypsy moth spread.

16-021

GENETIC VARIATION OF THE GYPSY MOTH, *LYMANTRIA DISPAR* COMPLEX (LEPIDOPTERA: LYMANTRIIDAE)

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Isozyme variation and genetic relationships among populations of the *Lymantria dispar* complex, having different geographic origin, were electrophoretically analyzed on the basis of 32 loci. Samples from Japan were found to be genetically more variable (average $H_c=0.13$) than those from Europe (average $H_c=0.09$) and North America (average $H_c=0.04$). The low values of Nearctic populations reflect founder effects involved in their origin. Populations from Europe and North America (*L. dispar dispar*) were genetically homogeneous (average Nei's $D=0.01$). The Japanese samples from Hokkaido (*L. dispar hokkaidoensis*) and northern Honshu Island (*L. dispar* subsp.) were more differentiated showing a D from *L. dispar dispar* of 0.09 and 0.10 respectively. A high genetic distance ($D=0.25$) was estimated between *L. japonica* (from central Honshu) and all the *L. dispar* samples tested. Lab crosses between these two taxa showed that their reproductive isolation is only partial; therefore *L. dispar* and *L. japonica* should be considered as semispecies. The evolutionary relationships among the members of the *Lymantria dispar* complex are analyzed and discussed.

16-023

ADULT REPRODUCTIVE BEHAVIOR OF ASIAN GYPSY MOTHS, *LYMANTRIA DISPAR* L. (LEPIDOPTERA: LYMANTRIIDAE)

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The reproductive activities of the Asian gypsy moth were studied in the Russian Far East, Central Siberia and Germany, where this strain has recently been detected. The timing of male Asian gypsy moth attraction to synthetic pheromone has a bimodal pattern: most males are attracted in the afternoon, but a second, smaller peak of activity occurs after sunset. Daytime temperature modulated the timing of daytime attraction but did not alter the timing of the onset of attraction following sunset. Female eclosion and mating also followed a bimodal pattern: a midmorning to midday peak followed by a smaller afternoon peak. These bimodal patterns allow temporal coordination of the male's ranging flight and the availability of emerging and pheromone-emitting females. These patterns of attraction and the similarity of mating behaviors suggest that the Asian, European and North American strains should interbreed freely in areas of contact or new sympatry.

Following daytime mating and before egg deposition, females usually flew from their mating site at dusk when the light level fell to ca. 1 lux. A few females, however, delayed their departure to the dusk of the second night following mating.

16-024

ECOLOGICAL EFFECTS OF *LYMANTRIA DISPAR* IN NORTH AMERICA

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Forests in eastern North America have been highly disturbed for over two centuries. In addition to anthropomorphic related disturbance, major catastrophic events like chestnut blight (*Cryphonectria parasitica*) have changed the composition and structure of the hardwood forest. The introduction of *Lymantria dispar* over a century ago has also resulted in pronounced changes in these forests. The genus *Quercus* dominates many forests in eastern North America, and as the preferred host of *Lymantria dispar*, a possible loss of dominance could be possible. However, data suggest that *Quercus* still maintains a presence in much of the forests of eastern North American, although at smaller scales, such as stand level, *Quercus* may disappear entirely. Generally, the changes caused by *Lymantria dispar* have more to do with affecting successional rate than species shifts. In three example areas: New England, the mid-Appalachians, and Michigan, *Lymantria dispar* appears to increase the rate of succession, accelerating species replacement processes, competitive relationships, and altering the relative species contribution, but having a minor effect on the overall species composition.

16-025

LYMANTRIA DISPAR : REGULATORY CONCERNS AND RESEARCH NEEDS
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For more than one hundred years, *Lymantria dispar* has been spreading and causing serious forest damage in north-eastern United States. During that period, the species has been of minor concern throughout its natural range in Europe, only causing local and occasional damage at the southern extremes of oak forests. In recent years, there have been increasing reports from the USA and Canada about a possible "Asian strain" of *L. dispar* being introduced from the Russian far east, with a wider host range, more aggressive behaviour and, most obviously, females capable of sustained flight. These reports have stimulated European countries to enquire whether such a strain might present a risk to European forests and to ask whether it has already been introduced to Central Europe.

A study of European literature on *L. dispar* indicates that the characteristics of the so-called "Asian strain" have long been known to occur in European populations, and recent RAPD-PCR analyses of European and Asian populations suggest that no new introductions have taken place. For the moment, therefore, the situation regarding *L. dispar* in the palaearctic regions appears to be unchanged and European countries do not see the need to adopt new quarantine measures. However, there is an urgent need to understand the characteristics of different populations and their geographic distribution in both the palaearctic and nearctic regions.

16-026

MANAGEMENT OF FOREST INSECTS IN PORTUGAL

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In Portugal forest ecosystems extend over 1/3 of the country and forestal industries make a significant contribute to the GNP. The main trees present are the cork oak, *Quercus suber*, the holm oak *Q. rotundifolia*, pines mainly *Pinus pinaster* and *P. pinea*, and eucalyptus, particularly *E. globulus*. Frequent insects to be found on oaks are, among others, the lepidopters *Lymantria dispar*, *Tortrix viridana*, *Malacosoma neustria*, *Eruproctis chrysorrhoea* and the buprestid *Coroebus bifasciatus*. Due to causes which are briefly analysed, insect population management is not intensively undertaken. In pine stands the processionary moth *Thaumetopoea pityocampa* is endemic, and outbreaks occur frequently. "Secondary" bark beetles, mainly *Tomicus piniperda*, *Ips sexdentatus* and *Orthotomicus erosus*, are sometimes recorded attacking standing trees, particularly in the vicinity of areas struck by fire. Cultural practices are normally undertaken, while pheromone baited traps have been used for research purposes only. Economic damage is caused to eucalyptus plantations by the borer *Phoracantha semipunctata*, against which control measures rely upon the use of trap trees. In 1991, a new species of an Encyrtid was discovered in Portugal. This rather efficient egg parasite has been cultered and small scale releases conducted. Another exotic pest, the curculionid *Gonipterus scutellatus*, has recently entered the country. In general, only small scale management of forest insect pests is practised. Although biological and biotechnological control methods look promising, in most cases the knowledge required for its implementation is still found in an incipient stage. To a certain extent, natural control by parasites and predators is in operation, probably as a result of a parsimonious use of insecticides so far made in forestry in Portugal.

16-027

A STRATEGY FOR FOREST HEALTH: ECOSYSTEM-BASED PREVENTIVE PEST MANAGEMENT

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In countries, where organized forest management systems have a long tradition, also knowledge of the sources of pest problems in manmade forests has early developed. Nevertheless, forestry concepts frequently neglected the principles of safety for economical reasons, thus being forced to apply pest control methods by which rather the symptoms of instability were suppressed than were its roots eliminated. The strategy of modern forest protection should be directed to a general prevention against the risks of pest epidemics. Therefore, detailed guidelines for a forest management aimed at risk reduction must be established, based on a profound knowledge of insect epidemiology.

It seems there to be a crucial prerequisite to thoroughly understand the causative principle of predisposing / eliciting factors. This would us enable to identify and localize risky situations in advance. Pest monitoring could then be focussed to such areas and forest management could specifically take care of concepts directed to damage prevention. The limitation of forest economy by ecological facts could be more precisely determined, quantified and from a longterm perspective considered in the planning procedures of forestry.

Assessment and manipulation of the epidemic-related predisposition of forests require intensified studies into the host plant / herbivore / antagonist tritrophic interactions as well as research into the ecosystemic processes by which they are determined. Thus, forest entomology should not only concentrate its interest on this topic; it also should link itself closer to forest ecosystem research. This could considerably contribute to a better understanding and counteracting of pest problems in forests under changing environmental and economical conditions.

16-028

FOREST PEST MANAGEMENT IN THE UK

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Commercial forestry in the UK is dominated by non-native conifers, plantations which contribute only 24% of our annual softwood timber requirements. Minimising the risk of pest introduction on imported timber and studying the ecology of native insects on non-native trees have therefore been important themes in the ecology and management of forest insects in the UK.

Current issues in Plant Health will be discussed in relation to Pest Risk Analysis using *Ips typographus* and *Lymantria dispar* as examples.

The management of an introduced pest will be illustrated by *Dendroctonus micans*, emphasising the importance of internal quarantine, surveys, tree resistance and biological control by an introduced predatory beetle. The possible use of kairomones to monitor for the establishment and distribution of the predator will be discussed.

Defoliating Lepidoptera will be used to illustrate the problem of managing native pests that have adopted non-native plantation trees as hosts. The role of site and stress factors in affecting tree susceptibility and the significance of variability within insect populations will be considered.

16-030

MANAGING FOREST INSECT PESTS IN THE UNITED STATES:
CHANGING ATTITUDES AND PRIORITIES

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Although the complex of bark beetles and defoliators that affect U.S. forests has not changed substantially, there has been a significant shift in how these pests are perceived and what actions should be taken to mitigate their impact. More emphasis is being directed at managing forest ecosystems rather than specific forest pests, recognizing that the disturbance caused by insects, diseases, and their interactions with environmental factors affects the health and stability of forests. There is much concern about the introduction of exotic pests and how increased international trade and travel might exacerbate this threat.

In the 1990s, the southern pine beetle, *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae), and the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) continue to challenge forest managers and property owners and are the subject of suppression and/or eradication projects on state and federal lands. The status and impact of these pests, and current approach to their management will be discussed in detail.

16-029

INTEGRATED CONTROL OF THE SPRUCE WEB-SPINNING SAWFLY, *CEPHALCIA ARVENSIS* PANZER IN ITALY, WITH PARTICULAR REFERENCE TO THE SILVICULTURAL ASPECTS (HYMENOPTERA: PAMPHILIIDAE)

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The spruce web-spinning sawfly *Cephalcia arvensis* caused a large outbreak in the period 1985-1992 in two areas of the Venetian Prealps (N-E Italy), concerning about 1,200 ha of 70 to 80-year-old plantations of Norway spruce (*Picea abies*).

Climatic, pedological, and silvicultural factors seem to be predominant among the possible causes of the outbreaks. In fact it has been possible to point out that: 1) the period 1983-1986 was in both areas unusually hot and dry, especially during the developmental time of the pest (June-July); 2) a strong water stress occurred in the three years preceding the outbreaks; 3) inside each area, the distribution of the insect attack was not uniform, reflecting the variability in soil conditions and in stand density.

The relationships between these factors and the insect attack may be interpreted in two complementary ways: 1) lower mortality and faster development of the insect induced by favourable climatic conditions (high summer temperatures and lack of precipitations); 2) variation in food quality as a consequence of the water stress suffered by the trees, according to the soil features and the stand density.

The IPM program applied against the pest was based on the following steps: 1) monitoring of adults, especially males, with visual traps and sampling of prepupae in the soil, 2) set-up of life tables and identification of key-factors of mortality, 3) definition of tolerance level of the pest according to the number of expected adult emergences, 4) direct control on the whole attacked area by preventing adult females to climb the trees by means of sticky bands put around the trunk of every tree. This control method allowed to reduce from 54 to 71% the number of eggs laid on treated trees with respect untreated ones. Two additional experiments were conducted on small plots using an insect growth regulator insecticide against larvae in the foliage and four strains of entomogenous nematodes against the prepupae in the soil. Two nematode strains gave mortality percentages around 50% and look promising for future applications.

16-031

FOREST INSECT PEST MANAGEMENT IN CANADA: PAST, PRESENT AND FUTURE

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There are about 119 million hectares of forest land in Canada managed for timber production. Most of the stands are in the Boreal Forest zone. The recognition of insect problems, and how these problems were addressed, is generally reflected in the intensity of forest management practiced. The native and introduced insect pest species were treated differently. Specific examples are given for the past and current insect management methods, and general trends are described for the future.

Up to the middle of this century no control measures were attempted unless the insect outbreaks covered extensive areas. This meant that the main focus of the earliest control measures were aimed at highly damaging bark beetles and defoliators. The methods used were suppression techniques, mainly the use of hard chemicals for defoliators, and burning and salvage logging for bark beetle-infested stands.

Classical biological control was, and still is, used against pests of foreign origin which were introduced into Canada without their natural enemies. Inspection and eradication are used to prevent or delay establishment of exotic pests.

At present, with the forest landbase and timber supply shrinking, there is a shift towards more intensive forest management. This change is reflected in methods of control - attempts are made to manage insect pest problems before drastic measures are required to control them. This change is also noticeable in the way insect problems are addressed. Besides defoliators and bark beetles in mature stands, insects attacking regeneration and affecting cone and seed production are also of concern. It is recognized that insect damage and its impacts should be minimized in an ecologically sound and environmentally friendly manner. Insects are managed instead of being suppressed, and whenever possible an integrated approach is taken. Broad spectrum chemicals have been replaced by the more selective microbial insecticide for defoliators. Silvicultural manipulation and pheromone-based management are currently being investigated as a means to minimize the impact of bark beetles.

In the future, attempts will be made to prevent insect problems before they have a chance to develop. The approach will be more holistic and ecologically based, taking into account other interactions besides those between the host tree and insect species. The control agents used to manage forest insects are likely to have narrow activity ranges - a requirement likely to be demanded by the environmentalists and general public. This will increase the pressure on scientists to develop novel control agents (such as genetically engineered microbes or introducing insect pathogenic microbes into host trees). However, some of these novel approaches are not likely to gain general acceptance, and the trend may be to manage forest insects through increasing the health of the forest.

16-032

MANAGING ATTACK BY BARK AND AMBROSIA BEETLES (COLEOPTERA: SCOLYTIDAE) IN FIRE-DAMAGED *PINUS* PLANTATIONS AND SALVAGED LOGS IN SOUTH-EAST QUEENSLAND

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In late 1994, bushfires in the Beerburrum area north of Brisbane damaged 8688 hectares of pine plantations (mostly *Pinus elliottii*, *P. caribaea* and *P. taeda*). A volume of 660,000 m³ of timber was considered salvagable. Large scale salvage operations were commenced quickly in order to minimise damage by the established exotic bark beetle *Ips grandicollis* Eichhoff and associated sapstaining fungi. The bark beetle began attacking fire-damaged trees 6 weeks after the fire and was significant in most areas at 10 weeks. Sapstain caused by *Ips* became significant at the completion of a life cycle of the insect (about 4 weeks in summer). Losses caused by *Ips* and sapstain during this phase were estimated at several million dollars Australian, most of this in privately-owned plantations where salvage was delayed for several months.

Salvaged timber is stored on a 34 hectare site at Beerburrum under water spray to inhibit degrade of logs by insect and fungi. Serious attack of the logs by the ambrosia beetle *Xyleborus perforans* (Wollaston) and decay fungi has occurred one year after the storage commenced. The circumstances associated with the insect attack and management options are discussed in this paper.

16-034

INSECTS AND MITES CAUSING GALLS ON THE LEAVES OF MAPLE *ACER PSEUDOPLATANUS* L. IN POLAND

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In the years 1994-1995 studies were conducted on the leaves of *Acer pseudoplatanus* L. in the Ojców National Park in Poland. Studies included a total of 4000 leaves randomly collected from 40 trees /100 leaves from each tree/. The species composition of galls was similar in both years. *Aceria pseudoplatani* /Corti//Acar., Eriophyidae/ turned out to be the most numerous species in both years, namely 2637 individuals and 3659 indiv. respectively. Index of occurrence frequency /F/ for the two years reached the highest value in *A. pseudoplatani*, namely 1.252 and 1.829 respectively, and the lowest in *Dasineura vitrina* /Kieffer/ /Dipt., Cecidomyiidae/ 0.013 in 1994. On the basis of Agrell's index of species coexistence it was shown that the strongest bond existed between *Driscina glutinosa* Giard /Dipt., Cecidomyiidae/ and *A. pseudoplatani*.

16-033

THE IMPROVED MANAGEMENT OF FOREST INSECT PESTS IN POLAND

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Pest insects are one of the major threats to the forests in Poland. They occur at various intensity in all stand age classes. Seedlings are attacked by *Melolonthinae* especially in plantations established at former agricultural lands. *Curculionidae* is the other group of pest in plantations and *Hylobius abietis* is the most common species. In mature stands *Hymenoptera* and *Lepidoptera* larvae as well as secondary pests mainly *Ips typographus*, *Tomicus piniperda* and *Phaenops cyanea* occur in the outbreaks.

Until recently pyrethroids have been used on a large scale in pest management. At present the quantity of pyrethroids is greatly reduced. Pheromone traps are applied as a protective measure against *H. abietis*. Recent research has shown that the application of the fungus *Phlebiopsis gigantea* to the stumps at clear-cuttings does not only protect trees against root rot but also inhibits the development of the *H. abietis* larvae in the stumps.

In the control of defoliators the *B.t.* products are used against the *Lepidoptera* and diflubenzuron against *Hymenoptera* pests. Secondary pest are reduced mainly by the removal of colonized trees and by the captures of *I. typographus* and *T. piniperda* beetles in feromone traps. Chemical insecticides are still applied to soil against white grubs and studies are carried out to develop biological methods for the control of that group of pests.

16-035

DESCRIBING THE NATURAL HISTORY AND ASPECTS OF THE ECOLOGY OF *CINARA CUNEOMACULATA* (APHIDINA; STERNORRHYNCHA)

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Cinara cuneomaculata (DELGUERCIO) is a lachnid tended by ants. It colonizes young shoots of the larch (*Larix decidua*). In 1995 the population-maximum was in August/September as typical for this species. It derived from annual sequence of generations and coherent fluctuations of abundance. The annual growth pattern of the host plant correlated with this late population-maximum.

A preview is given setting the results in relation to climatic change, year-ring structure of the host plant and larch-honeydew honey yields.

16-036

MORPHOLOGICAL DIAGNOSIS, DISPERSAL AND SEASONAL DYNAMIC OF *CINARA PRUINOSA* HARTIG (APHIDINA (LACHNIDAE)) SUCKING ON *PICEA SP.* IN BERLIN - BRANDENBURG DURING 1992 - 1995

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Cinara pruinosa lives throughout the year on different spruce trees (*Picea abies*, *P. bicolor*, *P. excelsa*, *P. pungens*, *P. schrenkiana*, *P. smithiana*, *P. sylvestris*) in Berlin - Brandenburg and reaches high density levels (600 - 1100 individuals on trees up to 2 m height). New results will be introduced about the morphological characteristics, the preferred sucking-places and the seasonal dynamic of *C. pruinosa* living on different species of *Picea*. Observations of direct visit of *Apis mellifera*, *Bombus hortorum*, *B. pratorum*, *B. terrestris* and *Paravespula germanica* indicate that *C. pruinosa* becomes more significant as a possible supplier of honeydew for Hymenoptera as assumed till now. Furthermore experiments with rings of glue demonstrate that the dependance of ants (*Lasius niger*, *L. fuliginosus*) of *C. pruinosa* is more important as assumed up to now.

16-038

Bark and ambrosia beetles of Costa Rica, density and occurrence with respect to distribution of major host trees in the La Selva Biological Station reserve.

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In conjunction with a major rain forest inventory projecy being conducted in northeast Costa Rica, we are carrying out a finer scale studies of the relationship of resource quality (host species) and resource abundance to the community structure of wood-boring beetles (Scolytidae, Platypodidae). In 1994 and 1995, trunk sections (baits) of totally eight species were placed out in primary and secondary rainforest, an abandoned cocoa plantation and a managed polyculture of native trees (replicates of each set of baits). After 25 days on the forest floor, they were mounted in rearing bags for approx. four months to collect the offspring beetles. More than 40,000 specimens of Scolytidae and Platypodidae emerged from the baits. There were considerable differences in emergence patterns, both within each habitat and between forest types. Fine-scale distribution of the species shows that most species aggregate substantially, with five species making about 90% of the total. The numbers are related to resource input as estimated by density/area of host trees.

16-037

THE IMPACT OF APHIDS ON THE GROWTH OF SITKA SPRUCE (*PICEA SITCHENSIS* (BONG.) CARR.) IN UPLAND BRITAIN.

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Sitka spruce is the most important timber producing tree in north and west Britain and over 530 000 ha have been planted with this species in upland areas. These plantations are prone, however, to defoliation by green spruce aphid (*Elatobium abietinum* Walker) (Homoptera; Aphididae) and infestation by root-aphids (*Pachypappa* and *Pachypappella* spp.) (Homoptera; Pemphigidae), and both types of aphid have the potential to reduce spruce productivity.

In 1992, the UK Forestry Commission started a long-term, manipulative field experiment in a recently re-planted area of Hafren Forest, Wales, to obtain quantitative information on the impact of aphids on spruce growth and to assess the economic consequences of damage. The experiment comprises four treatments in which insecticides are used to exclude *E. abietinum* or root-aphids separately or in combination, and a fifth treatment where *E. abietinum* populations are raised artificially. The influence of aphids on tree increment is estimated by comparing the performance of infested and uninfested trees.

Natural aphid populations have been low since 1992 and have had no significant effect on tree growth (n=180 trees), but *E. abietinum* on trees inoculated artificially in April 1994 (n=45 trees) attained densities 33 times higher, reaching, on average, 19 aphids.100 needles⁻¹ by June. This caused 41% of the 1991-1993 needles to fall prematurely, and led to a 22% reduction in leader shoot growth (p<0.001) and a 10% reduction in shoot growth on the upper whorl (p=0.013) by the end of the year. Total tree height was 12% shorter at the end of 1994 compared with naturally infested and sprayed trees (p<0.001). Results from 1995 indicate similar growth losses in the second year after defoliation.

16-039

STUDIES OF THE INTRASPECIFIC VAFIATIONS IN *TRICHOGRAMMA DENDROLIMI*, A MAJOR BIOLOGICAL CONTROL AFENT AGAINST THE PINE MOTHS IN CHINA

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Trichogramma dendrolimi is a widely distributed and the most frequently utilized parasitoid against forestry insect pests in China. Intraspecific variations of the parasitoid have long been speculated as one of the major factors responsible for its variations in control efficacy. In this study, 12 stocks of the parasitoid, collected from five species of *Dendrolimus* and 12 localities from 22°N to 43°N in China, were established. Comparative experiments of the following aspects were conducted between the stocks to reveal possible intraspecific variations: (1) 72 different combinations of cross trials between the stocks were conducted and revealed no evidence of reproductive isolation among the stocks. (2) Comparison of performance under a wide range of climatic conditions showed all the stocks responding similarly to favorable and high temperatures. However, the northern stocks performed better than the southern ones after cold storage. (3) Under some diapause induction conditions, the rates of diapause were significantly higher in the northern stocks than in the southern ones. (4) No significant differences were detected in the ability of host discrimination among the stocks. (5) When eggs of 6 host species were arranged into various host combinations and exposed to 11 of the stocks, no significant differences, either in host preference or host suitability, could be detected between the stocks. These results as a whole suggest that the populations of *T. dendrolimi* across China still remain one well defined species, but some intraspecific variations have occurred. Howcver, the observed variations were generally low and sometimes unstable, it is as yet difficult to take them into practical considerations in the utilization of this parasitoid.

16-040

THE INTRODUCTION OF *PAUESIA JUNIPERORUM* (STARY) (HYMENOPTERA: BRACONIDAE) INTO MALAWI FOR THE BIOLOGICAL CONTROL OF *CINARA CUPRESSI* (BUCKTON) (HOMOPTERA: APHIDIDAE)

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From 1986, the cypress aphid, *Cinara cupressi* (Buckton) was reported causing extensive damage to exotic and native cypress stands in Malawi. Since 1991, the Forestry Research Institute of Malawi (FRIM), with technical support from the International Institute of Biological Control (IIBC), has been undertaking a classical biological control project against the pest; this project forms part of a wider integrated pest management project funded by the British Overseas Development Administration.

In 1994 and 1995, the parasitoid *Pauesia juniperorum* Stary, collected from western Europe and screened at IIBC, UK, was shipped to Malawi for rearing and direct field release. The field releases were made in cages containing aphid ingested branches during the months August to early October. Parasitoids released in 1994 did not establish. However, in 1995, females released in cages provided a large number of progeny with an evenly balanced sex ratio. These were allowed to disperse naturally from the cages. Regular branch samples taken later in the year indicated that the parasitoid had dispersed several hundred meters from the cages. Mean parasitism on branches was 48 per cent.

16-042

THE IMPACT OF *PHYTOLMYA LATA* INJURY ON GROWTH AND BIOMASS ACCUMULATION OF IROKO (*MILICIA EXCELSA*)

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The Iroko gall fly, *Phytolmya lata* is the most important limiting factor to the establishment of Iroko plantations in Subsaharan Africa. We studied the effect of *P. lata* injury during Iroko early development on dry matter accumulation and partitioning. Losses in components of growth and dry matter accumulations were measured monthly and at the end of 18 months.

Overall, compared with control plots, growth of infested plots were significantly reduced. Plants infested were significantly shorter, had smaller number and smaller sized leaves. Average height and girth of the infested plots were reduced by 52% and 30% respectively. Dry weights of stem, branches and leaves were significantly greater in uninfested plots than in infested plots. Reductions in dry matter accumulations and partitioning at 18 months were 68.93% for stem, 48.32% for branches and 64% for leaves. Foliage of infested plants were highly chlorotic. We conclude that *P. lata* injury has significant effect on growth and biomass accumulation in the early development of Iroko plants.

16-041

ECOLOGICAL VARIABILITY IN THE POPULATION OF CURCULIONID PESTS OF FOREST PLANTATION (CURCULIONIDAE: COLEOPTERA)

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The curculionids form an important part of the phytophagous beetles and are associated with a variety of flora. A large number of curculionids are pest of forest trees of economic importance like *Tectona*, *Dalbergia*, *Eugenia*, *Pinus*, *Mangifera*, *Quercus*, *Shorea*, *Bombax*, *Ficus*, *Acacia*, *Tamarix*, *Cassia*, etc. The study has been made by observing the type of flora, of different regions for working out the possible co-relation of the concerned species with biotic and abiotic factors. Sympatric and Allopatric occurrence of the species has shown promising relationships which are likely to be proved extremely useful in establishing ecological patterns of distribution.

The various aspects which were studied to correlate their ecological characteristics with behavioural patterns pertaining to the amount of host plant range, seasonal prevalence, fecundity, development and population structure will be highlighted.

16-043

WOODWASP NATIONAL CONTROL PROGRAM IN BRAZIL

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Brazil has about 6 million ha of forest plantations of which 2 million ha consist of *Pinus* spp. Most of these stands were planted with a small number of species at high density and inadequate forest management. In 1988 an outbreak of *Sirex noctilio* Fabricius was recorded in Rio Grande do Sul State. It is present in 200,000ha, and is also advancing on Santa Catarina and Paraná States. The use of biological control is the best measure to control *S. noctilio*. The most effective agent is *Deladenus siricidicola* Bedding, a nematode that sterilizes the females. In 1989, it was bred by the PROGRAMA NACIONAL DE CONTROLE A VESPA-DA-MADEIRA (PNCVM). This programme is broad in scope and also includes: the early detection and monitoring of the spread through the use of trap trees and ground inspections, and the adoption of preventative measures, through adequate forest management. Plans for the future include the introduction of the parasitoid wasps *Rhyssa persuasoria* (L.) and *Megarhyssa nortoni* (Cresson) to compliment the nematode and the parasitoid *Ibalia leucospoides* (Hochenworth).

16-044

THE ROLE OF AIR AND SOIL POLLUTION IN THE DEVELOPMENT OF INSECT OUTBREAKS IN FORESTS

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Forest declines of a new type have occurred in many countries in Europe and Northern America. It has been debated, whether these are caused by pests or by pollution. In practically all cases insect pests or fungal diseases have been dominantly present and give sufficient reason for forest decline. The pollutant levels have only rarely reached such a degree, where trees have been damaged in experimental exposition. The conclusion has been drawn that pests are killing the forests, not the pollutants.

This conclusion may, however, be an oversimplification. The damage is clearly worst in the nearest vicinity of polluting industries and intensity of forest damage is regionally in clear positive correlation to pollution. Historically, forest declines have increased simultaneously with human population and industrialization.

It is possible to assimilate the contradictory thinking of forest entomologists and environmentalists. It is only necessary to accept the fact that pollutants and strong silvicultural measures may diminish the pest resistance of trees to a degree where a pest outbreak is released. In fact this has been known by forest entomologists for several decades. It is important to realize that insect outbreaks represent only the final phase of a multistress syndrome.

16-046

DEFOLIATION-INDUCED INCREASE IN WILLOW LEAF FLUCTUATING ASYMMETRY REFLECTS LOW FOLIAGE QUALITY FOR *MELASOMA LAPPONICA* (CHRYSOMELIDAE)

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We studied the interaction between the leaf beetle, *Melasoma lapponica*, and willow, *Salix borealis*, in the surroundings of the large nickel-copper smelter in NW Russia. Although foliar Ni reached 250 µg/g and ambient SO₂ - 250 µg/m³ near the smelter in 1994, growth, reproduction and leaf fluctuating asymmetry (FA) of willow did not indicate pollution-induced stress. However, moderate pollution ameliorated foliage quality in 1993, as indicated by higher survival of larvae fed with leaves from moderately polluted plots. This alteration of host-plant quality contributed to an outbreak of *M. lapponica* in moderately polluted sites, whereas in both heavily and slightly polluted habitats, beetle density was consistently lower. By the end of the growing season of 1993, *S. borealis* was almost completely defoliated in moderately polluted sites for the first time since we started our observations in 1989. During the following year, we recorded increases in leaf FA of *S. borealis* in the outbreak plots. Since FA in these plots was at the background level in 1992, before an increase in beetle density was observed, we considered the increase in FA as a response to defoliation. Willow leaf quality in the outbreak sites was lower in 1994 than in 1993, as indicated by higher mortality of beetle larvae, lower fecundity of adults and lower fertility of eggs. This decrease in host-plant quality was correlated with an increase in leaf FA. Accordingly, changes in plant quality caused by severe herbivore damage (a biotic stress) could be assessed by measuring FA, a non-specific stress indicator. We conclude, that our insect-plant system fits the Plant Vigour Hypothesis and possesses self-regulatory mechanisms based on negative feedbacks: plants stressed by herbivory are poorer quality hosts for the next generations of herbivores.

16-045

EFFECTS OF AIR POLLUTION IMPACT ON THE POPULATION DYNAMICS OF THE LESSER SPRUCE SAWFLY, *PRISTIPHORA ABIETINA* CHRIST (HYMENOPTERA: TENTHREDINIDAE)

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Pristiphora abietina is a severe pest of Norway spruce in plantations situated outside the natural range of the host tree. Causes of its outbreaks are mainly sought with faults of forest management, but also air pollution impact is suspected of triggering its epidemics. Theoretically, food chain balance at various points is vulnerable by pollutants. The results of field and laboratory studies strongly support the hypothesis that air pollution impact ranks with the factors which influence the population dynamics or even potentially provoke epidemics of this insect.

There is good evidence of food quality improvement by increased nitrogen supply to the host plant, partially by shifting the protein / phenolics ratio in the young needles, thus influencing larval growth. Sulfur concentration in the foliage obviously affects the mortality of the early larval instars, which - apart from the coincidence of bud break with oviposition - is in line with the degree of damage on the trees. Acidification of the soil contributing to the development of lots of needle litter, improves the resting conditions for the cocoons in the ground, thus reducing nymph mortality. Significant mortality factors attacking the resting nymphs in their cocoons are pathogenic fungi. Their efficiency is affected by the traits of soil litter and by the effects of heavy metals accumulated in the fungi. Finally, the deposition of sulfate and ammonium ions to the ground cover may be toxic for cocoon parasitoids.

In the light of these findings the persisting epidemics of *P. abietina* in spruce plantations appear as the result of combined environmental effects, the both being anthropogenic: forest management and air pollution.

16-047

DISAPPEARANCE OF MASS APPEARING PHYTOPHAGOUS INSECTS ON THE EXAMPLE OF PINE MOTH *Dendrolimus pini* L. (Lepidoptera, Lasiocampidae)

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At present a number of mass phytophagous insects become rare and disappearing. One of them Pine moth. Its vast outbreaks in Western Europe were stopped in the beginning of a current century, in East Europe - in a middle of a current century, in Siberia tend cessation at present. The displacement of outbreaks for east and their termination is connected to loss of the primary stands Scotch pine, having by a maximum stock of the needles. As have shown our researches, its complete disappearance connected with anticipatory fall of the needles called acid deposits. At loss more than 10 % falling Pine moth is absence. It is saved in the primary stands in common with enthomophages (Malyshev, 1994). The complete absence of a mass phytophagous species conceals in self the following dangerous phenomena. Clearing of a ecological niche of the consumers of the needles; ii Increase of threat of outbreaks as insects, as other organisms; iii Infringement forest successions. Thus, the complete absence of mass appearing phytophagous insects is result further disintegration of forest ecosystems. The prevention it can be served by measures on reduction anthropogenous press and preservation species diversity of forest ecosystems.

16-048

ACCUMULATION OF METAL POLLUTANTS IN RED WOOD ANTS, THEIR INFLUENCE ON THE COLONY DEVELOPMENT AND USING OF ANTS IN THE BIOINDICATION

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Considering environmental conservation and biomonitoring, it is important to note that some functional groups of red wood ant workers are loaded with the highest content of metal pollutants found among boreal forest animals (Nuorteva, 1990). The highest content of metal pollutants was recorded in all studies among foragers (outside workers) in the red wood ant colony. In artificial feeding experiments of ant colonies with metal contaminated honey (500 mg CdCl₂ per 1 kg honey) showed the rise of metal content of foragers up to 75 mg/kg Cd/dry wt. Extremely high metal content (up to 73.4 mg/kg Cd) was recorded also in foragers in a polluted industrial and mining areas of Europe (Stary, Kubiznakova, 1987). For comparison, in unpolluted Estonia and Finland regions this parameter was only 7,1 mg/kg, still lower Cd content was determined in reserve ants. Negligible amounts were found in females, males and in the brood, 0,1 - 0,25 mg/kg both in unpolluted and in polluted areas and in artificial fed colonies. Detail analyse of artificial fed ant colonies showed that Cd and Hg compounds inhibit the activity of enzymes participating in ATP synthesis (Migula et al., 1993) on which the formation of energy stores and the development of newly emerged workers into reserve ants is directly based. Therefore the lack of reserve ants in observed nests was to be expected. Particularly low level of metal compounds in brood and in sexuals gives evidence of the ability of the ant colony to protect their offspring through filtering poisons in the sociobiological food chain. Ants possess systems of physiological and sociobiological poison tolerance. This is one of the features offering singular possibilities of better survival for social insects, especially in the conditions of the superstructures like supercolonies.

The highest content of pollutants in foragers is very important appearance for using of red wood ants in the bioindication of environment pollution level especially in forest biocoenoses, whereas a red wood ant colony collect a lot of aphid honeydew and prey food during summer period (active season). **Through colony food chain also pollutants from vegetable and prey food accumulate during lifetime mainly in the foragers as eldest polyethic group of ants.** It is relatively easy to estimate by the help of foragers the pollution level of forest biocoenoses. It would be cheap express method for estimation of environment pollution situation

16-050

MOLECULAR AND PHYSIOLOGICAL BIOMARKERS IN INSECTS AS THE TOOLS IN ENVIRONMENTAL HAZARD ASSESSMENT

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The use of biomarkers in environmental pollution enables monitoring of stress responses, ranging from biochemical to community levels. The biomarker is defined as a biological response to chemical(s) that allows to measure its exposure and toxic effects. A good bioindicator system should be based on well recognised and combined methods developed in laboratory and later validated in the fields. Among biochemical biomarkers inducible detoxification enzymes with a low level of specificity (MFO, glutathion S-transferases) are good measures of specific stress factors. The use of patterns of enzymes involved in energetic transformations, metallothioneins or lipid peroxidation are better for evaluating pollution hazards from heavy metals. On the organism level changes in energy-yielding substrates and in the adenine nucleotide pool, with the adenylate energy charge are useful indicators of sublethal stress.

Weak and target points in the functioning of the temperate zone forest ecosystems under multistress from industrial pollution are well recognised. Among them small soil-dwelling arthropods, ants, carnivorous insects or spiders are of a special importance. Since measures on a molecular level are useful in monitoring for effects or exposure before alterations in the population or community level are recognised, their validity in environmental assessment are undoubted. Advantages and disadvantages as well as constraints in the use of biomarkers exemplified on social insects, herbivorous insects and spiders will be presented in details. Data obtained in laboratory conditions on insects exposed to controlled stressing factors could not be easily transferred to the field conditions. Biomarkers studied in ants receiving controlled excess of metals in natural conditions provided more information on that matter and made easier the extrapolation of individual biomarkers to the field monitoring at a community level.

16-049

DENDROCHRONOLOGICAL INDICATION OF RESISTANCE OF PINUS ELДАРICA MEDW. TO DIORICTRYA SPLENDIDELLA H-S (LEPIDOPTERA, TORTRICIDAE) IN GEORGIA

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It was made comparative dendrochronological study of two adjacent pine stands plots damaged and undamaged by *D. splendidella*. As a rule the separate trees and the damaged stands are forming wide year tree rings and containing the more resin canals in the one tree ring. The plants of rapidly growing stimulation by watering or planting on rich moisture soil cause the formation of the wide year rings and damage such trees by *D. splendidella* on 80-90%. After stopping the watering or when the main root run through the rich stratum soil deeply, the tree year rings become narrower and the pest starts gradually leave such trees and plots, and already don't settle on them.

Putting forward the conception of *P. eldarica* resistance depending on annual growth as a genetic property.

16-051

THE ROLE OF FOREST ENTOMOLOGY IN THE PROTECTION OF THE GLOBAL ENVIRONMENT

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Environmentalists have the task to protect the life supporting machinery of nature. Forests play an essential role in the functions of that machinery. The task of the forest entomologists is to prevent such human activities, which reduce the resistance of trees against their arthropod pests.

Forest insect calamities, promoted by environmental pollution and overdimensioned silvicultural practices, have caused forest deaths and decline on a local and regional scale. A danger of even more extensive damage is possible, because insects represent in nature a really mighty force. Their species diversity and biomass are superior to all other systematical categories of animals. If the damaging potential of insects is released in full, the result may be really horrible.

It is not safe nor rational to try to control insect calamities when they have already broken out. Prevention of such human activities, which have the capacity to promote outbreaks of pests, is a much safer method. Prevention needs, of course, a much deeper knowledge than the control of outbroken calamities. Here, in this congress, we have arranged two sessions in order to clear up the basis for the anthropogenic promotion of insect population outbreaks.

16-052

EDGE EFFECT IN THE DISTRIBUTION OF FOREST INSECTS AND ITS SIGNIFICANCE IN MONITORING AND SAMPLING

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Edge effect in the distribution of some gall-forming, phyllophagous, xylophagous and conophagous insects was studied across the larch forest/alpine meadow ecotone (Monte Bondone, Trentino, Northern Italy) and spruce forest/calcareous meadow ecotone (Bosco del Cansiglio, Veneto, Northern Italy) in 1993-1995. The occurrence of concrete species and ecological groups of dendrophagous insects was recognized by the numbers of damaged "food units" (1-year shoots, buds, cones, percentage of grazed and sucked needles) or by the numbers of "nests" (for *Epinotia tedella*, *Cephalcia arvensis*, and *Laspeyresia pactolana*) and galls (for *Sacchyphantes abietis* and *Laspeyresia zebeana*). It was shown that the damage by nonspecialized phytophagous insects was concentrated on young trees in meadow positions not far from the forest border, while specialized pests damaged the inner forest trees in near-border positions more intensively. In many cases, higher damage was not registered on the border-line trees exactly but into the limits of a more or less wide transition zone between forest and meadow. In this zone, all the types of damage occurred, although some of them were absent on core forest trees or on the isolated trees in meadow positions. Some insect species were probably attracted to border conditions (e.g. *D. abietella*, *L. zebeana*), while others avoided border trees and increased their abundance in core habitats (*S. abietis*, *C. arvensis*), or did not show noticeable preference (*E. tedella*, *L. pactolana*). During the period of observations, the last two species were characterized by relatively high population density. A practical conclusion is that both monitoring and sampling of forest pests at low population density have to take into account the general pattern of insect abundance across the forest border.

16-054

BLUE-STAIN FUNGI VECTORED BY AGGRESSIVE AND NON-AGGRESSIVE BARK BEETLES (COLEOPTERA: SCOLYTIDAE)

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Bark beetles are generally regarded as forest pests, but only a very small proportion of the 5,800 species in the world are aggressive and can kill healthy trees. Most species are non-aggressive, and colonize dead trees (saprophagic species) or severely stressed or dying trees (facultatively parasitic species). We have studied the blue-stain flora of several Eurasian bark beetles that colonize Norway spruce, including the aggressive *Ips typographus* L., the facultatively parasitic *I. duplicatus* Sahlb., *Pityogenes chalcographus* L. and *Polygraphus poligraphus* L. and the saprophytic *Hyurgops palliatus* Gyll.
I. typographus and *I. duplicatus* carried very high frequencies of the phytopathogenic fungus *Ceratocystis polonica* (Siem.) C. Moreau. The other bark beetles carried no known pathogens. We mass-inoculated Norway spruce with the fungi most frequently isolated from the beetles (*C. polonica*, *Ophiostoma piceae* (Münch) P. Sydow, cfr. *Ambrosiella* sp. and Dark sterile sp.A). *C. polonica* was the only fungus that stained the sapwood 15 weeks after inoculation (56% of cross-sectional sapwood area). It induced five times longer phloem necroses, 21 times more necrotic cambium and 11 times more necrotic phloem than any other fungus. These results are consistent with the hypothesis that aggressive bark beetles are associated with more phytopathogenic fungi than other bark beetles.

16-053

INVESTIGATIONS ON THE LIFE HISTORY OF *NEMOSOMA ELONGATUM* L. (COLEOPTERA: OSTOMIDAE), A BARK BEETLE PREDATOR

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The ostomid beetle *Nemosoma elongatum* is a common and widely distributed predator of bark beetles. The larval and adult stages prey on various scolytid species. In Central Europe the main prey species are the coniferous bark beetle *Pityogenes chalcographus* and the beech bark beetle *Taphrorychus bicolor*. *N. elongatum* uses the aggregation pheromones of the scolytids to locate prey. Therefore this antagonist was found in high numbers in bark beetle pheromone traps, especially in Chalcoprax baited traps. Detailed investigations on life history were initiated as a preliminary step in determining the role of this species in natural bark beetle regulation. In laboratory embryonic and postembryonic development depended on temperature and averaged 70 days at 25/15°C. Prey consumption of the *N. elongatum* larvae was approximately 30 bark beetles per individual. The adult longevity averaged 3 to 5 months and mean fecundity was 64 eggs per female at 20/10°C. At this temperature one *N. elongatum* individual consumed one bark beetle adult per day on average. Due to these data this predator species must be considered as an important and effective antagonist of bark beetles. The suitability of *N. elongatum* for biocontrol of bark beetles is discussed.

16-055

IPS TYPOGRAPHUS : EVIDENCE OF PASSIVE MOVEMENT TOWARDS OVERWINTERING SITES

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A proportion of the populations of *Ips typographus* overwinters into the bark of attacked trees, whilst the remaining part overwinters in the litter at the base of the trees. From samplings made during the winters 1991-92 and 1992-93 in several localities of southern Belgium, a strong correlation was established between the numbers of overwintering individuals per litter sample, and the weight of infested bark present in the sample. This suggests that the beetles have passively moved into the litter with falling pieces of infested bark. Repeated samplings on the same sites showed that this process continued throughout the winter. Management implications are discussed.

16-056

SELECTION CUTTINGS AND POPULATION DYNAMICS OF THE SPRUCE BEETLES IN HOKKAIDO

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After selection cuttings in Hokkaido, the spruce beetle (*Ips typographus japonicus* Nijima) attacks and kills standing spruce trees.

We studied the relationship between selection cuttings and the attacks of beetles for more than 10 years. Over five years we collected spruce beetles with pheromone traps at the same three places in the forest and investigated the relationship between selection cuttings and trap catches.

(1) Total number and the seasonal distribution of trap catches at the same place differed among years. However, in the year when the largest number of beetles were captured and which were large in body weight, not many trees were killed. (2) The number of beetles captured by traps in places where selection cuttings had taken place increased in the years after cuttings. (3) At log depots in the forest where cuttings had been done, a large number of newly emerged beetles were captured in the summer for several years after cuttings. Combined with other studies on the populations of spruce beetles, monitoring using pheromone traps will bring us some important information on the population dynamics of spruce beetles.

16-057

THE SUCCESSION OF BARK BEETLE DIVERSITY IN WINDTHROW AREAS

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In three Swiss windthrow areas of the 1990 winterstorm "Vivian" the development of the scolytid beetle complex was monitored. Each experimental area, formerly forests of mainly spruce trees, was divided into a cleared and an uncleared plot. Results of window trap catches from 1991 to 1994 are presented.

In the uncleared windthrows, there was an average augmentation of the species number by almost 50 % and a more than 6-fold increase of the number of individuals during the two first years after the storm. Thereafter, species as well as individual numbers decreased. Cleared areas exhibited the same species dynamics, but individual numbers steadily increased, though at a low level.

Compared to the situation in an adjacent intact forest, species numbers in the windthrow areas were always higher. Species and individuals in the uncleared treatment always outnumbered those in the cleared plots and the forest.

The most frequent species in all years was *Pityogenes chalcographus* (thereby causing no damage in adjacent forests, in contrast to *Ips typographus*). The proportion of species living on broadleaf trees decreased with time.

Generally, the Shannon-Weaver diversity index showed an increase with time. No differences were found between clearing treatments. Diversity was lowest in the intact forest.

16-058

INSECT COLONIZATION ON EXOTIC VS NATIVE FOREST PLANTATIONS: THEORETICAL CONTENT AND EMPIRICAL EVIDENCE

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Forest entomologists have long argued that plantations of exotic tree species are more prone to attack by native insects than are native tree species. Theoretical ideas on the rates of species colonization on novel plants are discussed. A review of the literature indicates that exotic tree species often have lower colonization by indigenous insects. This pattern is examined across a gradient from tropical to temperate forests. Colonization of exotic tree species by native insects requires considerable adaptation and consequently may require many decades to complete. The implications of these findings to the use of exotic tree species is discussed.

16-059

EXPERIMENTATIONS OF BIOLOGICAL CONTROL OF FOREST DEFOLIATORS WITH BACTERIAL PREPARATIONS

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The laboratory tests carried out by using the bacterial preparations - Ecotéch-Pro and Dipel-8L demonstrated the different sensibility of the caterpillars of *Lymantria dispar* and *Tortrix viridana* depending on the outbreak phase of both defoliators.

In the field conditions, the aerial spray with ULV treatments assured a high efficacy (96-98%), if is applied an optimal dose and is established the most favorable moment of treatment.

16-060

ASSESSMENT AND MONITORING PINE CATERPILLAR DEFOLIATION WITH AIRBORNE VIDEO

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Monitoring forest health with airborne video was developed by the USDA Forest Service for recent years. The airborne video system consists of two components-an airborne video image acquisition components and a video image processing component. Videography offers many advantages: lower cost than ground methods, higher accuracy than sketch mapping, and easy handling by users, meanwhile, the video data can quickly be converted to a digital form for analysis.

In China, monitoring pine caterpillar (*Dendrolimus punctatus* W.) defoliation in Jiangshan County, Zhejiang Province and suburbs of Nanning City, Guangxi Zhuang Autonomous Region using Y5 aircraft, combining video camera system with GPS navigation. The flying height is from 597m to 1900m, depending on the topography condition. The clear images were acquitted not only under clear weather but also under overcast weather. After video imagery has been captured on type, it can be viewing on monitor in Lab and processing using MIPS system. The results indicated that the 50% defoliation can be detected, the resolution is 1m to 2m. The combined use of GPS, GIS, and airborne video can provide geo-referenced spatial information about insect damage, and forest health managers are able to use it to detect and monitor forest pest over large area.

16-061

NANTUCKET PINE TIP MOTH, *RHYACIONIA FRUSTRANA* (COMSTOCK) (LEPIDOPTERA: TORTRICIDAE) SITE AND STAND RELATIONSHIPS IN EAST TEXAS

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Site and factors were evaluated to explore the relationship of the Nantucket pine, *Rhyacionia frustrana* (Comstock), stands of loblolly pine, *Pinus taeda* L., ranging in age from 2 to 5 years. Variables affecting infestation rates included depth of the soil A horizon; depth to the clay layer for rooting depth; height of pine; age of pine; and a ratio of the depth of the A horizon and soil texture. A series of discriminant function equations were developed to assist in evaluation of site and stand factors affecting Nantucket pine tip moth infestations.

Soil amendments of nitrogen and phosphorus and applications of herbicides affected Nantucket pine tip moth in two ways: applications of phosphorus tended to reduce tip moth infestations, although the sites were phosphorus-deficient; nitrogen tended to either slightly increase or have no effect. Herbicides provided a host that was more visible and these pines tended to have increased infestations. Infestation rates were related to site and stand factors and management of these areas should incorporate soil factors, tree species, time since planting, regeneration methods and soil and tree amendments.

16-062

A PRELIMINARY SURVEY FOR OCCURRENCE AND SPREAD OF THE PINE WOOD NEMATODES *BURSAPHELENCHUS* SPP AND THEIR POSSIBLE VECTORS IN PINE FORESTS IN ITALY

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A survey has been performed in Italy for pine wood nematodes of the aerial parts of pine trees to verify whether the quarantine organism *Bursaphelenchus xylophilus* (Steiner & Buhner) Nickle or other species of the genus are present.

Samples were obtained by removing portions of pine stems in an advanced state of decline or which had been dead for no more than a year. Among cerambycid species found on pine woody material collected, *Monochamus galloprovincialis* Olivier resulted until now the only carrier of nematodes. Data on the biology of this long-horned beetle in Central Italy as well as on its distribution are presented.

In all sampled areas of our country no specimens of *B. xylophilus* were found. *Bursaphelenchus mucronatus* Mamiya & Enda has been found associated with *M. galloprovincialis* adults and inside the wood of *Pinus pinaster* Ait. in three localities of Liguria; *Bursaphelenchus leoni* Baujard inside the wood of *P. pinaster* (Ravenna), *Pinus pinea* L. (Livorno) and *Pinus halepensis* Miller (Foggia); *Bursaphelenchus sexdentati* Rühm on *P. pinaster* (Ravenna). Italian populations of *B. mucronatus*, reared in malt extract agar plates on *Botrytis cinerea* Pers. ex Nocca & Balb., were examined by LM and SEM. DNA extracted from five pine wood nematode populations identified as *B. mucronatus* (Italy and France) and as *B. xylophilus* (Japan, USA, Canada) were compared by the random amplified polymorphic DNA technique (RAPD) based on the polymerase chain reaction (PCR).

16-063

DIVERSITY OF ICHNEUMONIDAE ASSOCIATED WITH GYPSY MOTH *LYMANTRIA DISPAR* IN DECIDUOUS FORESTS OF OHIO, USA (LEPIDOPTERA: LYMANTRIIDAE)

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Ichneumonidae have been collected from malaise traps in seven northeastern Ohio locations since 1989. The forests are predominantly mixed mesophytic hardwoods. The area has had a low density infestation by gypsy moth continuously throughout the sampling period. No significant defoliation has yet occurred, although three sites have been treated with *Bacillus thuringiensis* or gypsy moth nuclear polyhedrosis virus. Measurements of ichneumonid species diversity indicate greater consistency among sites within a single season than across several seasons. Ichneumonid species diversity displays a bimodal distribution with a major peak in early June and a second peak in August. Populations of genera that may parasitize gypsy moth (e. g. *Theronia*, *Pimpla*) appear to be stable from site to site and year to year. Application of virus or *B. thuringiensis* has had no discernible secondary impact on parasitoids, although *B. thuringiensis* had a significant impact on some nontarget Lepidoptera. This research has developed a useful baseline for ongoing studies of forest insect biodiversity in the midwestern USA.

16-064

GYPSY MOTH DEVELOPMENT ON FOLIAGE OF SEVERAL OAKS

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Gypsy moth (*Lymantria dispar* L.) larvae obtained from egg-clusters collected in two sub-populations at different gradation phases (culmination and latency) were fed in laboratory, at temperatures from 25 to 29 °C, on leaves of cork-oak (*Quercus suber* L.), holm-oak (*Q. ilex* L.), and pubescent-oak (*Q. pubescens* Willd.). 200 larvae, in groups of 50 per cage, were reared for each sub-population on each oak species. Cork-oak produced greatest survival rate (85 and 75%, respectively for sub-population in culmination and in latency phase), fastest development (ca. 30 days for males and 35 for females in both sub-populations), heaviest pupae (0.54 and 0.57 g for males; 1.28 and 1.45 g for females), and highest fecundity (304 and 369 eggs/female). Pubescent-oak produced lowest survival (56.5 and 42.5% in the two populations), longest development (38 days for males and ca. 43 for females), lightest pupae (0.36 and 0.31 g for male; 0.66 and 0.57 g for females), and lowest fecundity (128 and 66 eggs/female). Holm-oak was intermediate in its effects. Chemical analysis of foliage collected i) at the start of larval rearing, ii) when the larvae was at III instar, and iii) at VI instar, demonstrated significative quantitative and qualitative differences in phenol component but not in emicellulose, cellulose and lignin contents. In particular, the phenols were about 2-times higher in cork-oak and holm-oak (ca. 12% of dry weight) than in pubescent-oak (6-7% of d. w.) in the first and in second sampling. Gallic, p-cumaric and ferulic acids were predominant in cork-oak and holm-oak, gallic and p-hydroxybenzoic acids in pubescent-oak. A positive correlation can be supposed between the fenolic content and the gypsy moth larval development and fecundity. These results can partially explain the different gypsy moth density variations in the principal Sardinian oak regions. Cork-oaks are susceptible to periodic defoliation occurring every 5-6 years in forests interspersed with pastureland, and every 8-9 years in forests with the typical Mediterranean undergrowth. On the contrary, gypsy moth population remains sparse and rarely causes severe defoliation in holm-oak and pubescent-oak forests.

16-065

THE INFLUENCE OF THE MINERAL NUTRITION OF *EUCALYPTUS* PLANTS ON THE ATTACK OF DEFOLIATING CATERPILLARS

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This paper deals with the identification of different levels of boron on *Eucalyptus citriodora* plants which should influence the development of *Thyrinteina arnobia* (Lepidoptera, Geometridae).

The caterpillars were fed with *E. citriodora* leaves nutritionally characterized after different levels of boron and collected from the top and the bottom of the crown. The percentage of mortality of caterpillars fed with leaves from the bottom of the crown was higher than that of the caterpillars fed with leaves from the top the crown, probably due to the higher contents of boron on the bottom of the crown and consequently to the higher amount of phenolic compounds which act as growth inhibitor of insects.

16-066
ON THE RESPONSE OF *PHORACANTHA SEMIPUNCTATA* F. (COLEOPTERA, CERAMBYCIDAE) TO LOG TRAPS
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Lacking long distance pheromones, *P. semipunctata* perception of released compounds from the eucalyptus is vital for susceptible host detection, matting and oviposition. Based on this assumption the most common control measure against *P. semipunctata* during adults flight season, from South Africa to Europe and South-America, is the use of log traps which are very expensive and labour intensive when correctly applied. The aim of this work is to improve its effectiveness through better understanding the beetle response to host stimuli, in isolated pure stands of *Eucalyptus globulus*. Known to be strong flyers the beetle reveals a highly significant upwind short distance flight pattern (within the range of trap influence) and a predominant downwind behaviour in longer movements, over 2 kilometers. The placement of log traps is discussed considering the wind direction, orientation, topography and tree coverage.

16-068
DISEASE INTERACTIONS IN GYPSY MOTH POPULATIONS IN SOUTHWESTERN VIRGINIA, USA
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Southwestern Virginia is currently experiencing its first cycle of defoliation from the gypsy moth, which has recently invaded this area. As gypsy moth populations rise, natural enemies are moving into the area, including the gypsy moth nuclear polyhedrosis virus (NPV). NPV typically occurs at low levels until gypsy moth population rise; then, NPV becomes the dominant factor leading to gypsy moth population collapse. An intriguing new (to North America) disease of gypsy moth is the fungus *Entomophaga maimaiga* Humber, Shimazu, and Soper, which was introduced into SW Virginia by A. Hajek in 1991, and which is now widely established there. In 1995, we monitored gypsy moth populations in 33 woodlots near Lexington, VA, for the presence of the two pathogens. Gypsy moth populations in the woodlots varied from very sparse to high. Monitoring was most intense in the 5 blocks with the highest gypsy moth population density. Larvae were collected weekly, and those dying within 7 days were examined by light microscopy to identify the pathogen species involved. Also, burlap band larval counts were made weekly. The virus was strongly density dependent, being confirmed only from woodlots with higher density gypsy moth populations. In contrast, presence of the fungus was confirmed from gypsy moth cadavers found in woodlots containing sparse (less than 1 life stage found per burlap-band larval trap) gypsy moth populations. All higher density plots had extensive fungal induced mortality, but this failed to prevent a distinct second-wave NPV occurrence.

16-067
DEVELOPMENT OF MANAGEMENT PRACTICES FOR REDUCTION OF POPULATIONS OF THE INTRODUCED PEAR THRIPS, *TAENIOTHrips INCONSEQUENS* (UZEL) (THYSANOPTERA: THIRIPIDAE), IN SUGAR MAPLE FORESTS IN THE NEW WORLD
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Sap obtained from sugar maple trees (*Acer saccharum*) is used annually to produce over 11,000,000 liters of maple syrup, valued in excess of \$US100,000,000. A single moderate or heavy defoliation of a sugar maple tree by the pear thrips, *Taeniothrips inconsequens* (Uzel) (Thysanoptera: Thripidae), a periodic defoliator, results in the loss of the equivalent of one year's sap production over the next three years. Populations of pear thrips, a species which also attacks other valuable species of hardwood trees in the northeastern United States and eastern Canada, are regulated primarily through synchrony between expansion of new buds on the trees, the occurrence of female flowers, and emergence of female thrips adults from their over wintering location in the soil. The use of biological agents such as parasitoids (e.g., *Ceranisus* sp.), nematodes, and fungal pathogens (e.g., *Verticillium* sp.) has been or is being investigated to develop, where possible, intervention techniques for purposes of control. Routine use cannot yet be recommended for any of these agents with assurance, but both *Ceranisus* sp. and nematodes are expected to contribute to successful management programs. Prescribed burning, in limited trials, shows promise as a manipulable abiotic factor for control.

16-069
MANAGEMENT AND CONTROL OF FOREST INSECT PESTS IN CHINA
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The structure of Chinese organizations dealing with the research and management and control of forest insect pests is outlined. Accordingly, conifer and broad-leaved tree species are harmed by about 60 species of important insect pests. The most destructive pests in northern China are two cerambycids, *Anoplophora nobilis* and *A. glabripennis*, and in southern China pine caterpillar, *Dendrolimus* spp. The main reasons for outbreaks of forest insect pests are analysed. Currently, the research and practice of IPM are considerably popular in China. Especially more attention are paid for not only the pest control, but also the biodiversity protection. Present pest-control methods include the use of high-effective and low-toxic insecticides, biological control with NPV, Bt, *Beauveria bassiana*, and parasitic wasps, e.g. *Chouioia cunea*, *Trichogramma dendrolimi* and *Coccobius azumai* (Hymenoptera, Chalcidoidea) and the beneficial birds, and silvicultural methods such as selective cutting, with planting mixed stands etc. .

16-070

INCREASING TREE SPECIES DIVERSITY TO IMPROVE THE PEST CONTROL BY NATURAL ENEMIES IN FOREST MONOCULTURES: AN ORIGINAL DEVICE.

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Regarding sustainable forestry, there is a growing concern that monospecific forest would experience more severe or more frequent decays. The large availability of host plants and the weakness of the natural enemies control would render forest monocultures more prone to insects attack. But evidence to support this widespread belief is inconclusive, mainly by lack of properly formulated experiments.

A field device was thus developed in the Landes of Gascony, the largest unit of monocultural forest in Europe (1.2 millions hectares of pure maritime pine stands). Artificial plantations of broad-leaved trees, strictly constant in species composition and density, were regularly distributed within two areas of 1000 hectares of pure pine stands. The potentialities of these experimental plots as new ecological niches for parasitoids and predators is evaluated by comparison to control pine plantations of the same sizes, age and density.

Geostatistical methods used for estimating plant-pest-natural enemies interactions are described. First results concerning natural enemies diversity and spatial distribution of *Dioryctria sylvestrella* rate of infestation, according to the distance from broad-leaved stands, are discussed.

16-071

WITHIN-TREE DISTRIBUTION OF INFESTATIONS OF THE PINE NEEDLE GALL MIDGE, *THECODIPLOSIS JAPONENSIS* UCHIDA ET INOUE (DIPTERA: CECIDOMYIIDAE)Y. J. Chung, J. H. Lee¹, B. Y. LeeDivision of Forest Entomology, Forestry Research Institute, Seoul, Korea
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Efficient sampling units for estimating infestations of pine needle gall midge, *Thecodiplosis japonensis* Uchida et Inoue, on individual trees was developed from data collected in young Japanese red pine, *Pinus densiflora* Sieb. et Zucc., stands in 1992-1995. Samples of terminal and lateral shoots taken from the upper, middle, and lower crowns of different tree height classes (<2m, 2 to 4m, 4 to 6m, >6m).

No significant differences of infestations were found between terminal and lateral shoots, and among height class of trees. However, percentage of galled needles per shoot increases from the lower crown to the upper, and leader shoots are generally three times more than lateral shoots of the lower crown. Terminal shoots of mid-crown proved superior as a representative of tree infestations.

16-073

TOMICUS PINIPERDA IN NORTH AMERICA: CURRENT STATUS AND FUTURE PROSPECTS

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There are more than 370 exotic forest insects now established in North America. The pine shoot beetle, *Tomicus piniperda* (Coleoptera: Scolytidae) is one of the most recent arrivals in North America, being first reported in the United States in 1992. Because of its potential to damage pines, a federal quarantine was implemented in 1992 that restricts movement of pine material (including pine Christmas trees, pine nursery stock, and pine logs with bark) from infested to uninfested areas in the United States and Canada. As of January 1996, *T. piniperda* was known to occur in 8 US states and 1 Canadian province.

We will report on the life history of *T. piniperda* in North America, its interactions with native *Ips* bark beetles, and the host suitability of several North American conifers for *T. piniperda* reproduction and shoot feeding. Host species in decreasing order of preference were: *Pinus*, *Picea*, *Pseudotsuga*, *Larix*, and *Abies*. We will also discuss the efficacy of different trapping techniques for survey and control programs.

Another topic to be addressed will be the potential release of the predaceous clerid beetle, *Thanasimus formicarius*. Clerids from France were shipped to the United States in 1995 and are now being reared in a laboratory in Michigan. Studies are underway to evaluate potential non-target impacts by the European clerid on native natural enemies of North American pine bark beetles, such as the native clerid beetle *Thanasimus dubius*. Results from these studies will be presented.

16-072

SUSCEPTIBILITY OF JAPANESE RED PINE TO INFESTATION BY PINE NEEDLE GALL MIDGE, *THECODIPLOSIS JAPONENSIS*, IN KOREA

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The Japanese red pine, *Pinus densiflora*, is the main host-tree of the pine needle gall midge, *Thecodiplosis japonensis*, which is most threatening insect pest to pines in Korea. Methods of evaluating the midge damage are designed to provide information for detection, control, and preventive management, which based on the relationship between the damage levels and tree characteristics or stand/site variables.

The pine trees having narrow crown width and small crown volume in comparison with the stem volume were highly susceptible from the midge damage and it was considered due to insufficiency of sound needles for unit stem volume. The midge-caused tree mortality began to occur from the pine stands having 60 percent shoot mortality and increased rapidly from 80 percent. The major factors relating to the damage severity were ground covering with undergrowth, aspect, position of slope, stand density and slope. Comparative hazard-rating system of pine stands on the basis of shoot mortality were made by the discriminant analysis used the main environmental factors.

16-074

POPULATION DYNAMICS AND BEHAVIOUR OF THE
PROCESSIONARY MOTH *THAUMETOPOEA PITYOCAMPA*
(LEPIDOPTERA, THAUMETOPOEIDAE) IN PORTUGAL

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In the Iberian Peninsula, outbreaks of the processionary moth *Thaumetopoea pityocampa* are frequently recorded. This insect poses the most important problem regarding the phytosanitary protection of pine ecosystems. Stands of the maritime pine, *Pinus pinaster*, and of the mediterranean pine *P. pinea*, located in two different geographic zones, in southern and central Portugal, have been monitored, and data on the population dynamics of *T. pityocampa* obtained. Particularly, rates of mortality and development of the different instars, were determined. The behaviour of the larvae was also analysed, with emphasis on intraspecific competition relationships. The main parasitic species were identified, and their impact on the life cycle of *T. pityocampa* evaluated. Preliminary life-tables for *T. pityocampa* in Portugal were prepared. Estimates of absolute population densities were conducted by counting larval nests and egg masses at different times of the year, and from adult catches with pheromone baited traps. The data obtained will be correlated with the rates of defoliation observed, and thus contribute to the improvement of management strategies for *T. pityocampa*.
If invited speaker, indicate title of session and name(s) of organizer(s):

16-076

PEST MANAGEMENT OF *TORTRIX VIRIDANA* (L.):
MONITORING POPULATIONS WITH PHEROMONE TRAPS
(LEPIDOPTERA TORTRICIDAE).
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The green oak leaf roller (*Tortrix viridana* (L.)) is a serious forest defoliator widely spread in Eurasian regions, where its populations show temporary type outbreaks. A technique for monitoring populations trends based on the capture of males in traps would be a very valuable tool in the management of this pest.
Previous attempts carried out in Europe to use pheromone traps to provide information about the green oak leaf roller population density have yielded contrasting results and the potential of sex pheromone traps for providing an index of long term population changes, is not yet recognized.
For this reason from the last outbreaks found in Central Italy, populations of *T. viridana* have been monitored in Tuscany since 1986 in permanent sampling areas. The traps used were of the sticky type ("Traptest"). The different sampling areas have been combined in one cluster and the trend has been examined over a large area.
In this way the annual catches of males over a 10 - year period resulted well correlated with the egg-masses density in each subsequent year ($P<0,0001$; $r^2=0,722$). The strength of this relation suggest that the pheromone traps may be used to support the quantitative sampling technique.

16-075

DAY-DEGREE MODEL SHOWING EMERGENCE BY FOUR
SPECIES OF SEED CHALCIDS *MEGASTIGMUS SPP.*
(HYMENOPTERA: CHALCIDOIDEA)

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In Denmark four seed chalcids *Megastigmus pinus* Parfitt, *M. milleri* Milliron, *M. rafni* Hoffmeyer and *M. suspectus* Borries have been shown to infest seed of the four commercially grown firs: *Abies alba*, *A. normanniana*, *A. procera* and *A. grandis*. Emergence in the laboratory of the seed chalcids, was recorded at six different temperatures. Results were used to make a day-degree model, that can be used to predict the emergence of the seed chalcids in seed orchards or seed production stands. Emergence of seed chalcids in situ was found using emergence traps placed on the forest floor. These results show that seed chalcids emerge with about one week spacing between species in following order *M. milleri*, *M. pinus*, *M. suspectus* and *M. rafni*. In addition males are shown to be three or four days earlier than females.
Validity of the model is sought by comparing it with the in situ emergence data.

16-077

A MONITORING METHOD OF MOTH LARVAE (LEPIDOPTERA)
ON NORWAY SPRUCE BRANCHES
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In chosen monitoring sites ten terminal parts of branches (1 m long) are taken from the lower spruce canopy (up to 3 m) at the end of winter and then brought in plastic bags into the laboratory. Moth larvae or adults are obtained through photoelectors. After the branches dried up the weight and the number of all needles are determined.
This method possesses several advantages: a simplicity, a minimum impact on insect populations, a clearly defined phenological period, a repeatability of the methods and a comparability of the results. It also aims at obtaining all the species (pests as well as nonoutbreak species) occurring on spruce in this phenological period. Cutting off ten branches from each monitoring site every year and possible injury to moth larvae (especially those of Geometridae) in the course of handling the branches may be considered as its main disadvantages.
Argyresthia glabrata, *A. amiantella*, *A. bergiella*, *Batrachedra pimcolella*, *Chionodes electella*, *Dichelia histrionana*, *Pseudohermenias abietana*, *Epinotia nanana*, *Thera variata*, *Puengelera capreolaria* and *Hylaea fasciaria* are the most abundant and frequent species at the monitoring sites in the Pol'ana and Tatra Biosphere Reserves and the Malá Fatra National Park. The larvae burrowing in buds and shoots are more frequent at the sites in 1,000 m a.s.l. and higher. In contrast, the mining species are predominant at a lower elevation. The estimated number of needles on ten branches (one sample) was $517,623 \pm 112,689$ (mean value \pm standard deviation, $n = 52$).

16-078

PROTECTING LIVE DOUGLAS-FIR FROM INFESTATION BY THE DOUGLAS-FIR BEETLE USING AN ANTIAGGREGATION PHEROMONE

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Studies over a four-year period demonstrated the efficacy of an antiaggregation pheromone, 3-methylcyclohex-2-en-1-one (MCH), for protecting live Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, from infestation by Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins.

In 1992, MCH was applied to the perimeter of 1-ha circular plots at a rate of 60 g/plot. Multiple-funnel traps baited with frontalin, seudenol, 1-methylcyclohex-2-en-1-ol, and ethanol were located outside of but near the treated plots. The mean percentage of Douglas-fir host trees (≥ 20 cm DBH) that were mass-attacked was reduced by 80% within treated plots compared with untreated plots. However, there was a significant increase in the percentage of mass-attacked trees outside of the plots in the vicinity of the funnel traps. Therefore, in 1993, the efficacy of MCH alone was tested. The MCH was applied to plots ranging from 2.1 to 2.6 ha in size at rates of 45-76 g/ha. The percentage of host trees that were mass-attacked was significantly lower on the treated plots (0.2%) compared to the control plots (8.5%).

Dosage studies conducted in 1994 and 1995 determined that application rates as low as 20 g MCH/ha are effective in preventing infestation of high-risk trees.

16-080

THE POTENTIAL FOR INTEGRATED CONTROL OF *MINDARUS ABIETINUS* KOCH (HOMOPTERA:APHIDIDAE) IN *ABIES BALSAMEA* CHRISTMAS TREE PLANTATIONS.

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Balsam fir comprises the majority of Christmas tree sales in the northeastern and midwestern United States. For growers, the balsam twig aphid *Mindarus abietinus* Koch is a major pest because it causes needle distortion and/or loss which prevents the sale of trees for up to three years after attack. To date, there are few systematic methods used to monitor aphids. Growers have been found to apply pesticides at times too late to control pest damage but on time to pose risk to beneficial insects and breeding birds. A monitoring system is now being developed for growers to predict if and when control is necessary to prevent economic damage. A randomized split-plot design incorporating whole plot variables (mowing/fertilizer, herbicide/fertilizer, legume intercrop and no treatment) with subunit variables (systemic insecticide, insecticidal soap, water and no treatment) is also being used to determine the influence of such variables on numbers of aphids and their natural enemies.

16-079

FEMALE REPRODUCTIVE BEHAVIOUR AND PHEROMONE MATING DISRUPTION IN THE EUROPEAN PINE SAWFLY, *NEODIPRION SERTIFER*, (HYMENOPTERA: DIPRIONIDAE)E. Östrand, R. Wedding, O. Anderbrant, E. Jirle
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Females of the European pine sawfly, *Neodiprion sertifer* (Hymenoptera: Diprionidae) were released and observed in pine plantations treated with their sex pheromone, (2S,3S,7S)-3,7-dimethyl-2-pentadecanyl acetate, for the purpose of mating disruption, and in untreated areas within the same plantation. One experiment dealt with female reproductive behaviour and the other focused on the female's possibility to mate in areas treated with their sex pheromone (compared to untreated areas). In the first experiment, 30% of the females mated (n=127), during the release day in 1992 and 24% in 1993 (n=658). About 15% of the females mated more than once, and 4-9% of the females laid unfertilized eggs (parthenogenesis). About 55% of the females disappeared without having mated. After having mated, 60% of the females oviposited on the twigs, while 40% of the females disappeared. Predation was responsible for the majority of the disappearances, but also deliberate flight was noted.

In the mating disruption experiment about one third of the released females mated outside the treated area (n=258), but only 1-3% mated inside the area (n=295). About 50% of the females that mated stayed and laid their eggs on the twigs, and 50% of the females disappeared after mating. Significantly more females disappeared from their twig from inside the treated area than outside in both years. There was no difference in the proportion of unmated females ovipositing (2-12%) between the treatment and control. Possible different female reproductive strategies and the use of sex pheromone for mating disruption are discussed.

16-081

INTEGRATED MANAGEMENT OF *HYLOBIUS* WEEVILS IN POLAND

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Weevils of the genus *Hylobius* are the most destructive insect pests of reforestation areas in Poland. There are four species of *Hylobius* recorded in Poland, but only *Hylobius abietis* (L.) and *H. pinastri* (Gyll.) have the economic importance in forest plantations.

In Poland pine weevils occur every year on the whole territory of the country showing a continual tendency of growing in number.

Different methods are integrated to effectively reduce damage caused by pine weevils. This integrated management is aimed at protection of plantations against pest insects and keeping losses at a tolerable level. The following elements are integrated into a management: 1) silvicultural protection by soil scarification and delayed planting of trees, 2) reduction of weevil abundance by mechanical (freshly cut billets of Scots pine and baited ground traps with synthetic attractants) and biological (stump deterioration by fungus *Phlebiopsis gigantea* (Fr.: Fr.) Julich) methods, 3) chemical protection of seedlings with contact and systemic insecticides, where the pine weevils cause great losses.

16-082

SUSCEPTIBILITY OF THE MORE IMPORTANT FOREST PEST INSECTS TO INSECTICIDES IN POLAND

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The susceptibility of forest pest insects such as *Lymantria monacha* L., *Dendrolimus pini* L., *Panolis flammea* Schiff., *Diprionidae* and *Hylobius abietis* L. to the used insecticides (pyrethroids, arylpropylether, acylureas, diacylhydrazine and preparations based on a bacteria *Bacillus thuringiensis*) was evaluated under laboratory conditions.

Two methods of evaluation were used. topical application and exposure of insects to treated food (pine twigs with or without needles).

Studies showed that although the selection processes of resistant populations to the frequently used insecticides (mainly to pyrethroids) are developed, the above mentioned insect populations can be effectively controlled with pyrethroid insecticides. The studies also showed that the examined insect populations are fully sensitive to acylurea and diacylhydrazine insecticides and biological preparations *B. thuringiensis*.

However, the sensitivity of insect species controlled to the used chemical and biological insecticides should be checked.

16-084

OBSERVATION ON A NEW DANGEROUS PINE SCALE- *INSULASPIS PINI* (MASKELL) -AND ITS CONTROL

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Through faunistic investigation from 1990 to 1994, *Insulaspis pini* (Maskell) was found out for the first time in Dalian Area and some other provinces and cities in China. It causes damages on some pine species, including *Pinus thunbergii*, *P. tabulaeformis*, *P. densiflora*, and *P. sylvestris* var. *mongolica* and also some fir species. The insects aggregate and suck at the bases of the pine leaves. Damaged leaf turns yellow at the beginning and then wilts and falls down to the ground. If the damage is serious, the whole plant will die. Its occurrence is usually accompanied by *Matsucoccus matsumurea* Kuwana. External morphological characteristics of shells of both sexes, adults, egg, prepupa, 1st-instar larva and 2nd-instar larva are described. Moreover, its life cycle and habits are reported. In addition, the design and application of a chemical control method is discussed.

16-083

NATURAL EMEMY ON THE BLACK PINE BAST SCALE (*MATSUCOCCUS THUNBERGIANAE*) IN COAST AREA OF KOREA

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The black pine bast scale(*Matsucoccus thunbergianae*) that has been damaging the black pine of southern coastal areas of Korea. It has one generation a year, comming out of the pine bark to mate and lay eggs from early March to early May. Especially, its peak time is from late March to mid-April.

The observed natural enemies of black pine bast scale were 9 species of Birds, 1 species of Amphibian, 32 species of Insecta, and 23 species of Spiders.

Especially, *Parus majoy minor* and *Paradoxornis webbiana fuwicauda* among the Birds, *Velinus nodipes* and *Coccinella septempunctata* of Insects, *Agriope amoena*, *Lycosa suzurii* and *Lycosa pseudoannulata* of Spiders species arc frequently observed in the research areas.

16-085

THE RELATIONSHIP BETWEEN THE GALL WASP, *ANDRICUS MUKAI-GAWAE* (MUKAIGAWA) (HYMENOPTERA: CYNIPIDAE) AND THE GALL INHABITING MOTH, *OEDEMATOPODA* SP. (LEPIDOPTERA: STATHMOPODIDAE)

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Gall parasitism by moth larvae in the unisexual galls produced by *Andricus mukaigawae* (Mukaigawa) on *Quercus serrata* in Nose, Japan was surveyed by dissection of the galls. Almost all moth larvae found in the galls were *Oedematopoda* sp. The gall parasitic moths did not significantly affect the survival of *A. mukaigawae*, though 53.4 % of the galls examined were attacked by the moths. Larval feeding habits of *Oedematopoda* sp. were examined under laboratory conditions. *Oedematopoda* sp. larvae were able to feed on *Q. serrata* leaves, but preferred unisexual gall of *A. mukaigawae*. Furthermore, almost all the larvae fed on artificially exposed *A. mukaigawae*, although the results of gall dissection indicate that some of the *Oedematopoda* sp. larvae became predators of the gall causers under natural conditions. Thus, the sclerenchymatous wall of the larval cell and its surrounding woody tissues protect the gall causers in *A. mukai-gawae* galls from attack by moths.

16-086

OBSERVATIONS ON *EUCALLIPTERUS TILIAE* (L.) AND *TINOCALLIS PLATANI* (KALT.) (RHYNCHOTA APHIDIDAE) POPULATION DYNAMICS IN MEDITERRANEAN ENVIRONMENT

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Eucallipterus tiliae and *Tinocallis platani* are pests of the urban shade trees. They infest in high numbers respectively limes and elms causing the rain of copious quantities of sticky honeydew.

Observations carried out in the urban avenues of Sassari (North-Sardinia) have annually shown for both species two peaks of abundance: the former between the late spring and the early summer, the later at the end of autumn. The two species had an annual alternation in the oviparae density (*E. tiliae*: 0.2 oviparae/leaf in 1994 and 3.05 in 1995; *T. platani*: 0.7 in 1994 and 7.57 in 1995) and in egg abundance (*E. tiliae*: 0.002 eggs/bud in 1994 and 0.29 in 1995; *T. platani*: 0.005 in 1994 and 1.34 in 1995). The same alternation occurred in the spring infestation peak timing, earlier (late may-early june) when there were many eggs otherwise about a month later. However the maximum density achieved by the aphids was strongly affected by the climatic conditions. For example, in 1994 the *E. tiliae* population growth was slowed down by an early leaf marginal chlorosis and subsequent scorching of the affected tissues caused by a limited spring raininess (only 98 mm in march-may). Every year the aphid populations rapidly declined in numbers in the early summer with few or no aphids left from half july to half october. This phenomenon seem strongly related to the changes in the food quality (particularly the limes in july always showed leaf scorching). On limes and elms, several predators (Aranea, Miridae, Anthocoridae, Chrysopidae, Coccinellidae and Sirphidae) were observed but their maximum abundance (0.36-0.53 predator number/leaf) was recorded only after one or two weeks from the aphid spring peak.

The population dynamics of *E. tiliae* and *T. platani* seems to be similar in the urban environment. The Mediterranean climatic conditions, characterized by dry summers, heavily influence their abundance trend which appears different from the one recorded in areas with greater raininess and cooler summers.

16-088

VARIATION OF THE FREE AMINO ACIDS PROLINE AND ALANINE AND LIPIDS IN *IPS TYPOGRAPHUS* (COL., SCOLYTIDAE) DURING DISPERSION

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Dispersing adults of the European spruce engraver *Ips typographus* (L.) were caught with pheromones traps and unbaited flight barrier traps, which were placed 5 and 10 meters away to catch imagines which did not respond to the lure. On trap trees, which were placed 50 meters in the main wind direction, in front of the pheromone taps, landing beetles were collected too. The beetles were frozen in liquid nitrogen immediatly after being trapped or collected.

Alanine, proline and glutamate constituted about 83% of the free amino acids found in *I. typographus*. According to a metabolic scheme the ratio of proline and alanine is understood as a measure of the actual energy stage, increasing values indicating an enhanced level of fuels for flight.

In *I. typographus* caught with barrier traps a proline alanine ratio of 1:2 (males) and 1:1,6 (females) was found, whereas corresponding values in adults from pheromone traps were 1:3.7 (males) 1:3.3 (females). Beetles collected just after landing had a ratio of 1: 2.4 (males and females). A proline alanine ratio of 4,3:1 was found in freshly emerged beetles.

Males and females from barrier traps had the highest average lipid content 620 (+/- 152) and 550 (+/-185) nmol acylester/mg dry weight, respectively. The average fat content of beetles collected after landing was about 25% lower and adults from pheromone traps showed 45% less fat. It seemed, that landing was not only due to a certain reduction of the fat body, but to a specific proline alanine ratio.

16-087

EVALUATION OF A BINOMIAL SAMPLING PLAN FOR HEMLOCK WOOLLY ADELGID

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The hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae) (HWA), is an introduced pest of eastern hemlock, *Tsuga canadensis* in the eastern United States. Because of the insect's small size, estimates of population density require cutting twigs from trees and examining the twigs under the microscope in the lab; a very labor intensive effort. If the dispersion of HWA can be described by an empirical distribution, estimates of population density may only require a count of the proportion of samples where the pest density meets or exceeds a threshold of 1. Therefore, the objective of this study was to determine if a single empirically derived distribution would satisfactorily describe HWA dispersion in both the spring (progredien/sexupara) and fall (sistens) generations in both Pennsylvania and Virginia, USA. To accomplish this, twenty trees were sampled in both Pennsylvania and Virginia. Six twigs were selected from each of four branches from each of the sample trees. The twigs were examined with the naked eye for presence of HWA. If present, the number of HWA were counted with aid of a microscope.

A linear relationship between the number of adelgids and the proportion of branches with adelgids was significant for both states and generations. Taylor's Power Law was used to compare these relationships quantitatively. A homogeneity-of-slope model indicated that the state-generation combination significantly affected the HWA dispersion ($p < 0.0001$). Since a single distribution did not satisfactorily describe the four dispersions examined, then a separate distribution would have to be estimated for each new application of the sampling procedure. Until it can be determined what is causing the differences in dispersion, a standard binomial sampling method cannot be applied for estimating HWA populations throughout its geographic range.

16-089

JUNIPER WOODLAND (*JUNIPERUS THURIFERA* L.) OF MOROCCAN ATLAS MOUNTAINS AND THE PEST IMPACT ON THEIR NATURAL REGENERATION.

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In Morocco, *Juniperus thurifera* (L.) grows up to 3000 m above sea-level. On account of its ecological value and social and economic role, this mountain tree is of great value, but is currently subject to intensive damage. Natural stands have been reduced by about 90%. Such losses can be attributed to the interactions of numerous factors. Among these, the impact of arthropods (mites and insects) which attack berries and seeds, appear to be one of the factors restricting the natural regeneration by reducing the number of seeds liable to germinate.

Investigations carried out in different juniper woodlands had the following objectives: (1) creation of a checklist of pest arthropods associated with female reproductive organs; (2) the detection of a phenological relationship between pest attack and the various steps of fruit development; (3) the identification of parasitic species associated with berry and seed pest species.

The investigations confirmed that arthropods can be damaging. The management and restoration of juniper woodlands require further investigations on the biology and ecology of pest arthropods and their predators such that a suitable control method can be devised.

16-090

MASS OUTBREAK OF *IPS DUPLICATUS* SAHLBERG, (Coleoptera, Scolytidae).

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Since 1992, the largest recorded outbreak of *Ips duplicatus* has been observed in the Czech Republic. This species spread here on approx. 1500 km² in the territory of north Moravia and Silesia.

It is commonly considered as a boreoalpine species, occurring in northern taiga from Sweden to north Sachalin and in solitary Alps localities. Because of planting monocultures of Norway spruce in unnatural conditions during last two hundred years, *Ips duplicatus* has enlarged its range from the beginning of this century. In connection with its spreading to warmer areas and with weather conditions in 90th years, *Ips duplicatus* has markedly shown its polyvoltine ability what resulted in rapid increasing its population density. *Ips duplicatus* seems to be even more aggressive than *Ips typographus*; mainly primary attack of healthy trees was observed. In area of the most infested forest districts, about 800000 m³ of spruce timber (infested mostly by *Ips typographus* and *Ips duplicatus*) were cut in the last years; we estimate that approximately one third of that is due to the attack of *Ips duplicatus*.

Classical control methods are tested during the years of the outbreak as well as known components of aggregation pheromone of this species. *Ips duplicatus* does not react to laying tree traps. Looking for attacked trees is very difficult because of no colour change of crown before emergence of beetles. Ipsdienol used in our experiments caused attack of first beetles on both cut and standing trees where they then produce their natural aggregation pheromone, which results in a heavy infestation of trees. Adding of the second known component of their pheromone - E-myrcenol - will increase attractiveness of a pheromone bait.

16-092

THE ROLE OF TERPENOID VARIATION AND PRODUCTION OF MIXTURES IN MEDIATING HOST TREE - HERBIVORE - PATHOGEN INTERACTIONS

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Multiple selection pressures from herbivores, pathogens, and plant-plant interactions have resulted in adaptations favoring mixtures of terpenoids that are highly effective against herbivores and pathogens. These same selection pressures also have resulted in numerous types of variation in the production of terpenoids. These include tissue-dependent induction vs. constitutive defenses, within-individual variation, among-individual variation within a population, developmental variation, and among-season variation. Terpenes are known to facilitate the deployment of other chemical defenses, to function in defense as mixtures and synergistically with primary nutrients, and perhaps lead to nonpreference due to their absenece. Abiotic stress ameliorates the regulating effect of mixtures and patterns of variation often leading to significant increases in herbivores and pathogens. Studies reported on reinforce the premise that in natural and managed systems, variation and diversity in phytochemicals are important to maintaining sustainable ecosystems.

16-091

EFFECT OF PINE NEEDLE TANNINS ON THE ACTIVITY OF TRYPSIN AND GUT PROTEASES OF *DIPRION PINI* (L.)

(HYM., DIPRIONIDAE)

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Pine needle (*Pinus sylvestris*) tannins were extracted from 10 trees in total. Proteases were isolated from guts of 5th instar larvae of the sawfly *Diprion pini* (L.) (Hym., Diprionidae) a defoliator highly adapted to pine needles. In protease bioassays benzoyl-arginyl-p-nitranilid (BAPNA) was used as substrate. Increasing concentrations of procyanidins in the needles correlated positively with the precipitation of bovine serum albumine and the inhibition of purchasable trypsin. Gut proteases were inhibited by pine needle tannins, too, but a linear correlation with the concentrations in the needles could not be observed. A crude extract of pine needle tannins was separated in different fractions by column chromatography on Sephadex LH-20 and a stepwise elution with 20%, 30%, 40%, 45%, and 50% acetone/H₂O (vol/vol). Gut proteases and trypsin are inhibited by tannin fractions in a different manner.

16-093

AN INTRODUCTION TO THE FOREST INSECT FAUNA IN LIAONING PROVINCE

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Liaoning is located at the northeastern part of China. Its flora is a combination of that from Changbai Mountain, North China and Mongolia; so the forest insect fauna is also complicated. According to our collection of 266,722 insect specimens, 2,595 species have been identified. They belong to 19 orders, 142 families and 1,463 genera. Among them, 7 orders, 48 families and 141 species are main forest pests; 11 orders, 40 families and 361 species are natural enemies of the pests and 72 species can be used in pest control. According to the taxonomic position of the pest species, and their distribution, we divided this province into 5 geographical and ecological regions:

1. East Liaoning medium and low mountainous region, important pests are: *Colephora laricella*, *Dendrolimus superans*, *Dryocosmus kuriphilus*, etc. 2. East Liaoning peninsular hilly region, important pests are : *Dendrolimus spectabilis*, *Cryptorrhynchus lapathi*, *Parathrene tabaniformis*, *Hyphantria cunea*, *Matsucoccus matsumurae*. 3. Northwest Liaoning windy and sandy region, important pests are: *Dendrolimus tabulaeformis*, *D. spectabilis*, *Pyrrhalta aenascens*, etc. 4. West Liaoning hilly region, important pests are: *Dendrolimus tabulaeformis*, *D. spectabilis*, *Anoplophora glabripennis*. 5. Middle Liaoning plain region, important pests are: *Cryptorrhichus lapathi*, *Paranthrene tabaniformis*, *Cossus cossus mongolicus*, ect.

16-094

ON FOOD PREFERENCES OF *CALVIA QUATUORDECIMGUTTATA* AND *SOSPITA VIGINTIGUTTATA* (COLEOPTERA COCCINELLIDAE) IN ALDER WOODS OF SOUTHERN ITALYV. Palmeri, A. Russo¹, S. Longo¹

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In alder woods of Calabria, several species of phytophagous insects are present; some of them as *Crypturaphis grassii* Silvestri (Homoptera Aphidoidea), *Psylla cordata* Tamanini (Homoptera Psylloidea) and *Galerucella solarii* Burlini (Coleoptera Chrysomelidae) are very common and, often, in high population densities. Associated with their pullulations, two species of lady-bird *Calvia quatuordecimguttata* L. and *Sospita vigintiguttata* L., are frequently collected; researches have been realized in order to investigate their food preferences. During 1989-90 and 1991-93, biological observations have been done in field and in laboratory. In the first two years, development of larvae respectively maintained with adults and nymphs of *C. grassii* and *P. cordata* and with eggs and larvae of *G. solarii*, have been studied while in 1991-93 the abdominal contents of *C. quatuordecimguttata* and *S. vigintiguttata* adults collected on woods have been observed. During 1989 mortalities of 93.3%, 50% and 100% have been recorded for larvae of *C. quatuordecimguttata* alimented respectively with *C. grassii*, *P. cordata* and *G. solarii* as far as these recorded for *S. vigintiguttata* have been 85.7%, 25% and 100% respectively. In 1990 only *S. vigintiguttata* have been bred and the recorded mortality percentages have been 50% on *P. cordata* and 100% on *C. grassii*. The development of the two ladybirds has been faster on *P. cordata* (a media of 20.88 days from egg to adult for *C. quatuordecimguttata* and of 23.78 days for *S. vigintiguttata*) than on *C. grassii* (a media of 27 days from egg to adult for *C. quatuordecimguttata* and of 26 days for *S. vigintiguttata*). Inside of the alimentary channel of adults of the two species of coccinellids only phragments of *P. cordata* have been detected.

16-096

ATTACKS OF BARK BEETLES ON DOUGLAS FIR IN ITALY

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The douglas fir *Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii* at present is widely cultivated in Italy mainly along the Apennines. Together with the diffusion of that rapid growth conifer a rising pressing of several indigenous phytophagous insects were observed.

Preliminary results of an ecological and faunistic survey in Douglas fir stands of different ages, showed that some indigenous bark beetles are able to attack this new host, particularly in young stands with trees stressed by prolonged drought.

The detected species at present are: *Cryphalus piceae* (Ratzeburg), *Cryphalus abietis* (Ratzeburg), *Pityogenes quadridens* (Hartig), *Pityogenes bidentatus* Herbst, *Pityophthorus pityographus* (Ratzeburg), *Xyloterus lineatus* (Olivier) and *Hylastes* sp.

16-095

GROWTH OF *CAMERARIA OHRIDELLA* Deschka&Dimić (LEPID., LITHOCOLLETIDAE)N. Dimić¹, Lj. Mihajlović², M. Vukša¹, P. Perić¹, S. Krnjajić¹, M. Cvetković¹¹Institute for plant protection and environment, Belgrade, Yugoslavia²Faculty of forestry, Belgrade

Leaf miner of wild chestnut, as a new insect species, was proved for the first time in the region of Ohrid in 1985. As a rule its occurrence is mass, and temporary defoliation of wild chestnut occurs, with a series of harmful effects for plant and also for the environment.

From the moment of its discovery the investigations on *C. ohridella* growth has lasted ten years continuously. Preliminary investigations were carried out in Sarajevo (1985-1992), exclusively under the strictly controlled natural conditions (the species has not occurred in the region of Sarajevo so far). In the region of Belgrade the growth of *C. ohridella* was observed from 1993 to 1995 in the several localities in nature and afterwards under the controlled natural conditions.

C. ohridella is a monophagous species, living only in the leaf of *Aesculus hippocastanum* L.

The growth of *C. ohridella* is very specific. In the whole area of distribution, the species has three generation per year. Eclosion of the moths of the 3rd generation with a part of the populations of the 1st and the 2nd generation occurs in the course of April 1st in June and 2nd generation from the end of July to mid August. The species overwinters in pupal stage in cocoon inside the pupae mine, in fallen leaves. Besides the pupae of the 3rd generation, the pupae of the 1st generation (26% on the average) and the 2nd generation (45% on the average) remain in diapause.

16-097

SPRUCE CONE INSECTS IN SWITZERLAND: ABUNDANCE AND EFFECT ON SEED PRODUCTION

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Cones from 29 spruce stands located in the five main regions of Switzerland were collected in 1989 and 1990. Cones of the first year were incubated and the emerging insects collected and identified. The regional abundance of spruce cone pests and a parasitoid genus (*Torymus* spp.) was studied. In cone samples from both years, the numbers of full, empty and damaged seeds were counted. Damaged seeds were classified according to the respective source of damage. The effect of the seed feeding insects on seed production was analysed.

From 14'000 individuals collected, 11 conophagous and seminiphagous species were identified. *Kaltenbachiola strobis* was the most frequent species, amounting to 40 %, followed by *Torymus* (29 %) and *Cydia strobilella* (26 %). The regional abundance showed a non-uniform pattern for the different species. Number of insects per cone were highest in the alpine region, with a maximum of 5.5 individuals in the South Alps.

Incidence of Seed Feeding insects averaged 71 % (1989) and 63 % (1990) of the cone crop, reducing the total number of full seeds by 27 and 19 %, respectively. *C. strobilella* as the most important pest caused seed losses of up to 48 % per cone. A multiple regression model was used to explain the variability in yield of full seeds, estimating the effect of seed feeding insects as well site characteristics. The resulting function indicates that loss variability depends on regional differences and site characteristics rather than on impact of seed feeding insects.

16-098

SPATIAL DISTRIBUTION AND SIGNIFICANCE OF SOME JEWEL-, LONGHORN- AND BARK BEETLES (COLEOPTERA: BUPRESTIDAE, CERAMBYCIDAE, SCOLYTIDAE) ON NORWAY SPRUCE [*PICEA ABIES* (L.) KARST.]

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The beetle fauna associated with Norway Spruce in Central Europe has already been well recognized. In 1987-1992 the very characteristic assemblages of jewel-, longhorn- and bark beetles were understudied in the thick (diameter 5-10 cm) branches of Norway Spruce trees (some 200 years old) growing singly on mountain meadows of Central Slovakia at an elevation of 1,300-1,400 m a.s.l. Four beetle species were frequent and abundant on spruce in such ecosystems, namely: *Anthaxia quadripunctata*, *A. helvetica* (Buprestidae), *Callidium aeneum* (Cerambycidae) and *Pityophorus ptyographus* (Scolytidae). Although developing together in the same branch (diameter 2-10 cm) with closely packed annual rings, particular species require special microhabitat conditions. Main factor affecting their occurrence is insolation. If insulated, the dying branch offers good conditions for development of the larvae of both *Anthaxia* species, mainly in its upper part, temperature of which may be some 50 °C during hot summer days. A side part of a branch is usually colonized by *C. aeneum*. Not insulated (moist, much cooler) bottom part is then a characteristic habitat for *P. ptyographus*. Imagines of both *Anthaxia* species are flower visitors. This probably affects their vertical distribution within a tree. The number of *Anthaxia* specimens is the highest in the lower branches and decreases towards the tree crown, whereas the numbers of *C. aeneum* and *P. ptyographus* are increasing. In both *Anthaxia* species and *C. aeneum* development takes two years, *P. ptyographus* has one year life-cycle. Although not economically significant, the species have a high bioindicative value in determining the health of Norway Spruce trees in mountain areas. Mainly the black *Anthaxia* species contrasting with yellow flowers which they rest on, often in high numbers near the dying trees, should be given more attention in forest conservation programmes.

16-100

BARK BEETLES (COLEOPTERA, SCOLYTIDAE) AND THEIR FOREST IMPORTANCE IN ESTONIA

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Over 13500 specimens of *Scolytidae* collected since 1850 are present in the insect collections of Estonia. Examination of the collections gives evidence of that the Estonian bark beetle fauna comprises 61 species. In addition, the relevant literature reviewed gave the data for the distribution of 9 species.

All species of bark beetles distributed in Estonia are insects of woody host plants. They are often regarded as serious tree killers, but virtually only a few species have economical importance as pests in forests. Most of bark beetles infest dead or dying host material and can be appreciated considering the species diversity in forest ecosystems.

Ips typographus is the scolytid of the greatest forest importance in Estonia. Several large-scale outbreaks have occurred in Norway spruce (*Picea abies*) stands after windthrow, snow-breakage, drought or fire during the last two centuries. *Tomicus piniperda* and *T. minor* are the most important bark beetle species in Scots pine (*Pinus sylvestris*). *Dendroctonus micans* is not numerous in spruce forests but since 1971 severe outbreaks have occurred in about 20-years-old plantations of Scots pine on sub-optimum sites. In reforestation areas damage are caused by *Hylastes cunicularius*. *Trypodendron lineatum* is the main species in felled conifer stems and logs.

During recent years 5 bark beetle species have been reported from Estonia for the first time: *Hylesinus varius*, *Hylurgus ligniperda*, *Scolytus mali*, *S. scolytus* and *Trypodendron proximum*.

16-099

SPATIAL AND TEMPORAL DISTRIBUTION OF *PITYOKTEINES SPINIDENS* (COL.: SCOLYTIDAE) IN THE HOST TREE. VARIATION OF THE SEX-RATIO DURING THE ATTACK PERIOD

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The study was carried out in the Silver fir forests of Valle de Arán (East Pyrenees), at 1,300 m, during the period October 1990 - June 1991.

The attack and colonization period of *P. spinidens* generation that emerges in summer can be extended until November, just before the adults begin overwintering.

The estimated total population in the trunk (1-7 m, 345 dm²) and during the maximum colonization period was 10,300 adults, with 25 % males. The maximum density was found at 4.7 m of height (17 cm diameter) with 9.3 males/dm² and 29.2 females/dm².

During the period of study a variation in the sex-ratio was observed, because of polygamy and the reemergence of the parent adults, characteristic behavior in this group of Scolytidae. In the first attack phases the sex-ratio of the parent adults reached values of 45-55 % males. In the maximum colonization period (October-March) the sex-ratio was 23-28 % of males. After the overwintering (March-June) the sex-ratio reached values of 30-35 % males.

16-101

XYLOTRECHUS VILLIONI (VILLARD) (COLEOPTERA: CERAMBYCIDAE), A PRIMARY BORER OF CONIFERS IN JAPAN: LOW DENSITY OF POPULATION AND HIGH DURABILITY OF SCARS ON THE STEMS CAUSED BY LARVAL BORING

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Xylotrechus villioni (Villard) is possibly the largest species of Clytini (Cerambycinae), and as a primary borer, its outbreaks rarely cause severe damage on *Abies* and *Picea* forest stands in Japan. The larva makes a rather straight gallery more than 1 m in length from the oviposition site to the "whirl-like gallery", in the center of which the pupal chamber and adult exit hole are made, and these galleries cause scars on the stems. In forest stands of *A. firma* (Tokyo and Kyoto Prefectures) and *A. sachalinensis* (Hokkaido), straight and whirl-like scars on the stems show highly cumulative damage on the trees, and whirl-like scars are distributed towards the bottom part of the stems. The spatial distribution of scars suggests that there is a random distribution of larvae as the larvae never aggregate and there is no interference between individuals. As "whirls" can be present on the stem for 30 years after the formation of the gallery, the density of existing mature larvae is very low. In an *A. homolepis* stand (Nagano Prefecture), cumulative damage on major branches suggests that there are almost simultaneous multiple larval borings with a very low survival from larvae to adults mainly due to the predation of picid woodpeckers.

This species is thought to be an unusual primary borer in that it infests conifers with heavy sap flow, usually has a very low density, and still affects tree appearance for a long time.

16-102

BIOLOGY AND ECOLOGY OF *CURCULIO* (*CURCULIO*) *PROPINQUUS* (DESBROCHERS) (COLEOPTERA: CURCULIONIDAE) IN SOUTHERN ITALY.

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At the present there is any information on the biology and ecology of *C. propinquus* and incomplete data on the host plants. Observations on this weevil were carried out on different species of *Quercus* present in Apulian Region (Southern Italy) during 1995. Adults and larvae of *C. propinquus* feed on the glands but the most harmful ones are the larvae which can destroy up to 100% of fruits of some Oak plants. In the woods of *Q. trojana* Webb and *Q. pubescens* Willd. the first species is the most damaged and between *Q. frainetto* Tenore and *Q. cerris* L. the glands of the first one are the most attacked. In the laboratory they also develop in chestnuts.

In glands the parasites are rare and, so far, only two have been found: *Scambus calobatus* (Gravenhorst) (Hymenoptera: Ictneumonidae) and the fungus *Metharizium anisopliae* (Metch.) Sorok. (Deuteromycotina: Hyphomycetes).

16-104

SOME BIOECOLOGICAL ASPECTS OF THE *ONCIDERUS IMPLUVIATA* (GERMAR) (COLEOPTERA: CERAMBYCIDAE) FROM *ACACIA MEARNsii* WILD IN THE NORTHWEST OF PARANA, BRAZIL

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Onciderus impluviata (Germar, 1824) is a very important pest species, being considered as a limitant factor for the cultivation of *Acacia mearnsii* Wild.

The objective of this work is the study of some bioecological aspects of this insect in a research stand of *A. mearnsii* in the Northwest of Parana, Brazil.

Falled branches with characteristics of slits made by the pest were collected, randomly, and kept in cages in the same place where they were picked.

The period and percentage of emergence of *O. impluviata* were checked during 1994 - 1995.

The emergence of adults occurred from October to January, with the peak in November and December. After the observation, the infested branches were destroyed and entirely crumbled.

16-103

LIFE TABLE STUDY OF THE TEAK BEEHOLE BORER, *XYLEUTES CERAMICUS* (WALKER) (LEPIDOPTERA: COSSIDAE) IN NORTHERN THAILAND

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The teak beehole borer is the most serious pest of teak in northern Thailand. To analyze the factors responsible for population trend, four life tables for the pest insect were constructed in 4 localities with different level of population density. In the low population site, heavy mortality occurred on the egg and 2nd-3rd instar larvae, while the mortality of those stages was remarkably low in the high population site. The predation by ants was found to be the major mortality factor for eggs and young larvae. About 99 % of hatchlings was disappeared due to dispersion and predation by ants at all sites. Mortality due to undetermined factors during the period of mature larval and pupal stages was relatively low at all sites. Key factor analysis showed that predation by ants during the egg and the 2nd to 3rd larval stages would govern the population changes of the teak beehole borer. The frequency by ant species was quite different between high and low population sites. In the high site, ants were not observed on 46% of trees, while they were not on 21% in the low. *Tetraponera rufonigra*, an important predator of the 2nd-3rd instar larvae, was not observed in the low population site.

16-105

GODASA SIDAE (FABRICIUS) : TAXONOMIC POSITION AND MORPHOLOGY OF THE DIFFERENT STAGES OF DEVELOPMENT.

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Godasa sidae causes severe damage to *Mansonia altissima* an indigenous tree species used for forest regeneration in Cameroon. However, this Lepidopteran has not received adequate attention by entomologists and consequently has been transferred from a family to another. This article deals with the systematic position of the species, together with preliminary studies on its morphological characteristics, including the description of larval chaetotaxy. *G. sidae* belongs to the family of Noctuidae, subfamily of Aganainae.

16-106

SEXUAL ATTRACTION IN THE PINWOOD NEMATODE

BURSAPHELENCHUS XYLOPHILUS

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Sexual attraction in the pinewood nematode, *Bursaphelenchus xylophilus*, was studied on agar strips in petri dishes. Males were attracted to virgin females but not to 4th-stage female larvae, mated females or gravid females. Males were responsive to female secretions emitted into agar and vaporizing substances from virgin females. Attraction of females by males was also noted.

No evidence of homosexual attraction was observed in either males or females.

Both male and female attractants seem to be volatile as well as water-soluble in nature

16-108

GEOGRAPHIC VARIATION IN ESTERASE ISOZYME PATTERN OF THE JAPANESE PINE SAWYER, *MONOCHAMUS ALTERNATUS* (COLEOPTERA: CERAMBYCIDAE)A.Yamane¹, R. Iwata², M. Natori¹, P.K. Chang¹, H. Makiha-

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The Japanese pine sawyer is well known as the vector of the pine wood nematode, *Bursaphelenchus xylophilus* (Nematoda; Aphelenchoididae) causing severe mortality of pines in not only Japan but also in neighbor countries. *M. alternatus* distributes widely in eastern Asia. Morphologically Japanese sawyer is not different from ones from mainland of China and Taiwan, except in color, but physiologically some of the latter do not need diapause before pupation. To detect genetic difference among the sawyers of various localities, the esterase isozymes of the sawyers were separated by polyacrylamide gel electrophoresis. It was clear that Chinese populations had different isozyme patterns from those of Japan, where also some grouping of the local strains was possible according to the isozyme pattern.

16-107

SAMPLING CHESTNUT FRUIT INSECT PESTS ON DIFFERENT CULTIVARS

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During two years (1994-95) studies were carried out on three tortricid moths, *Pammene fasciana* (L.), *Cydia fagiglandana* (Zeller) and *C. splendana* (Hübner), and a weevil, *Curculio elephas* (Gyllenhal), in a chestnut area of Southern Italy. Pests populations were sampled on two cultivars, "Tempesta" and "Ufarella", characterized by early (second ten days of September) and late (second ten days of October) ripening, respectively.

Damaged fruits and husks, number of larvae of the different species and plant phenology were detected.

P. fasciana (Early Chestnut Tortricid Moth) did not cause significant losses (10-12% of the young husks) in both cultivars. Larvae of this species were not found in the ripe fruits.

Large amount of *C. fagiglandana* (Middle Chestnut Tortricid Moth) larvae were collected from the fruit of both cvs, but in higher number on "Tempesta". *C. splendana* (Late Chestnut Tortricid Moth) larvae were more present on "Ufarella".

Only few *C. elephas* larvae were collected on fruits of "Tempesta", while high population densities were observed on "Ufarella".

The egg-larval parasitoid *Ascogaster quadridentatus* (Wesmael) (Hym.: Braconidae) emerged in a similar percentage (19-23%) from the tortricid larvae collected on the different cultivars.

The early ripening cv partially avoids the damage by *C. splendana* and completely escapes the attack by *C. elephas* larvae. The late ripening cv is more exposed to the injury of the chestnut fruit insect pests complex.

These observations have a practical impact when setting rational control strategies.

16-109

IN VITRO INDUCTION OF THE DISPERSAL FORMS OF PINE WOOD NEMATODE, *BURSAPHELENCHUS XYLOPHILUS* (NEMATODA: APHELENCHOIDIDAE)

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When the pine wood nematode, *Bursaphelenchus xylophilus*, parasitizes pine tree, they vigorously propagate in the resin duct of the pine, become the dispersal third stage juvenile (DSJ3) after the tree wilt and become the dispersal fourth stage juvenile (DSJ4) just before and/or just after riding on vector insects belonging to genus *Monochamus*. This process was reproduced *in vitro*. Each of 1,000 nematodes was inoculated on cultures of an identified fungus isolated from a tylenchid nematode, *Contortylenchus genitalicola* parasitic to *Monochamus*. When the cultures were kept in desiccator at 25 °C for 180 days, number of nematodes became 47,249 + 20,276 (mean + SE, n=8) and 78.1 % of the nematodes were DSJ3. When the cultures containing DSJ3 were added 0-day-old adults of sawyer, *M. alternatus*, 40.8% of the nematodes became to DSJ4. When the cultures were added last instar larvae, pupae or 10-day-old adults, rates of DSJ4 were 0.1, 5.0 or 5.7%, respectively. It was concluded that desiccation induces formation of DSJ3 and presence of vector insects, especially newly emerged adult, induces formation of DSJ4.

16-110

THE APHIDS LIVING ON *PINUS MUGO* TURRA IN ITALY
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Researches carried out in the latest years on the aphid fauna of mountain pine (*Pinus mugo* Turra) in Italy are here summarised. This pine has a relatively rich aphid fauna in the Alps and particularly in the Oriental Alps where the entities recorded from that conifer tree belong to Adelgidae with one oligophagous species (*Pineus pini* (Macquart)) and to Aphididae Lachninae with seven monophagous entities (*Cinara carnica* Binazzi, *C. covassii* Binazzi, *C. montanicola* (Börner), *C. neubergi* (Arnhart), *C. setosa* (Börner), *Eulachnus alticola* (Börner), *E. intermedius* Binazzi). Some other aphids were only occasionally found on the mentioned host-pine. Fewer species, on the contrary, were found on the Apennines where only some mountain pine groups are nowadays existing which are the remaining ones of the ancient anathermic postglacial peak formations. In these mountain pine residual scrubs only three *Cinara* species were recorded (*C. neubergi*, *C. pinea* and *C. pini*) together with three *Eulachnus* entities (*E. alticola*, *E. brevopilosus* and *E. intermedius*) some of them being linked on the Alps to *Pinus sylvestris*. In this regard and basing on the different sharing of the species between the Alps and the Apennines, aphids can give useful informations in order to have a better understanding of the history of the aphid-host pine coevolution.

Section 17

Tropical Entomology

17-001

INTEGRATED APHID MANAGEMENT IN COWPEA PRODUCTION IN NIGERIA

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Aphids, especially *Aphis craccivora* Koch, is usually a problem in the cultivation of the cowpea, *Vigna unguiculata* (L.) Walpers which is a dietary staple throughout tropical Africa. Non-chemical methods which have been investigated for aphid management in cowpea, specifically, cultural control, use of resistant cowpea varieties and biological control are highlighted. Combinations of these non-chemical methods into integrated pest management packages for the cowpea aphid are suggested and discussed.

17-002

THE CONTRIBUTION OF TRAP CROPS IN THE INTEGRATED CONTROL OF INSECT PESTS OF COTTON IN UGANDA.

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In the tropical countries the more traditional approaches to farming utilize polycultures. Population densities of herbivorous insects are frequently lower in vegetatively diverse habitats. A study on the insect pests-predators interaction on cotton associated with common farming crops in Uganda was conducted at each of Namulonge and Serere Agricultural and Animal Production Research Institutes in the two successive cotton seasons 1994/95 and 1995/96 respectively. Cotton pest and predator population densities on cotton as well as the surrounding trap crops; sorghum, maize, beans and malakwany were estimated weekly throughout the cotton growing seasons. Although the growing season of all the trap crops used in this study was relatively short compared to cotton, except that of malakwany, but they showed considerable attractiveness to major cotton pests, e.g. *lygus* to sorghum, *Helicoverpa armigera* Hb. to maize, whiteflies and jassids to beans and stainers to malakwany. At the same time the population of predators was relatively higher on cotton/trap crops than on cotton/control throughout the seasons. Seed cotton yields also were relatively higher in the cotton/trap crops (up to 20 %). Therefore, the contribution of the trap crops to integrated pest management of cotton pests in smallholder farming system is considerable.

17-003

TRITROPHIC INTERACTIONS IN THE MANAGEMENT OF *SPODOPTERA EXIGUA* IN CELERY

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In the USA, celery is an important vegetable crop, with values exceeding \$300 million annually. *Spodoptera exigua* (Hübner) is the major pest. *Liriomyza trifolii* (Burgess) is an important secondary pest, often occurring at damaging levels when its parasitoids are killed by pesticide applications aimed at *S. exigua*. Thus, pesticides or other control strategies are needed to control *S. exigua* without affecting the parasitoids. Attempts to use host plant resistance against these pests have been unsuccessful due to the presence of the carcinogenic linear furanocoumarins. In fact, a number of other potential pest management practices have been shown to increase the levels of these undesirable compounds. Unfortunately, simply tolerating the pest is not a viable option, as feeding by *S. exigua* reduces marketability and can induce production of the linear furanocoumarins. Therefore, a series of experiments were designed to evaluate the interactions between 1) plant chemistry, 2) *S. exigua*, 3) *L. trifolii* parasitoids and, 4) *Bacillus thuringiensis*. Based on these data an IPM program has been developed and evaluated in both experimental celery plantings and on a large-scale commercial crop. The results are encouraging; an economic analysis indicated that the IPM program produced better net profits while minimizing the potential for environmental damage, pesticide resistance, or human health concerns from linear furanocoumarins.

17-004

INSECT PEST MANAGEMENT OF SOME TROPICAL PERENNIAL CROPS

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Practical application of insect pest management strategies in large plantations of oil palm (50,000 ha.), cocoa (5,000 ha.) and Robusta coffee (2,000 ha.) are described.

Bag worms (*Mahasena corbetti* Tams., *Metisa plana* Wlk., *Cremastopsyche pendula* Joannis and *Clania* spp.) and nettle caterpillars (*Setora nitens* Wlk., *Setothosea asigna* van Eecke, *Birthissea bisura* Moore, *Darna trima* Moore and *D. catenatus* Snellen) were the main defoliators of oil palms in South-east Asia. The strategy to manage these pests was based on studies of their life history, dispersal pattern and natural enemies. Main emphasis was on prevention of pest outbreaks through strengthening the natural enemy complex by encouraging certain flowering herbaceous plants, and controlling the pest population in its early stage of establishment. Natural mortality was determined. Higher natural mortality indicated the pest might be controlled naturally. If natural mortality was inadequate, treatment was deemed necessary. Known pathogen of the pest was augmented to control the infestation. If pathogen was not known or was inadequately effective, insecticides were used sparingly.

Cocoa trees, temporary shade trees (*Gliricidia*) and permanent shade (coconut palms) constitute the cocoa crop system. The main pests of cocoa in Sumatra were *Helopeltis* spp. and *Zeuzera coffeae* (Sn.). Recently (1994) the cocoa pod borer has become established. *Brachyplatys* sp. and *Ectropis* (*Boarmia*) sp. on *Gliricidia*, *Hidari irava* (Mr.), *Rhynchophorus* spp. and *Oryctes rhinoceros* L. on coconut were endemic pests. *Helopeltis* was controlled by manipulation of black ants (*Dolicoderus bituberculatus* Mayr.). In places where black ants were not established infested pods were treated with insecticides. *Z. coffeae* was controlled by spraying suspension of *Beauveria bassiana* on the canopy. The cocoa pod borer was somewhat restricted by the black ants. *Brachyplatys* was controlled by augmentation of its egg parasites while *Ectropis* and *H. irava* by augmentation of respective virus. *Rhynchophorus* was managed by bait trapping.

The most serious insect pests of coffee in East Java (Indonesia) were *Planococcus citri* (Risso) on branches and *Hypothenemus hampei* (Ferr.) on berries. The former was controlled by manipulating conditions conducive for the natural enemies and the latter by controlling off season berries and spraying *B. bassiana* to infect the adults.

17-006

Convenient Synthesis and Field Activity of Isomers 2,8-Dioxa-1-ethyl-3,5,7-trimethylbicyclo[3.2.1]octane, A Male-Produced Aggregation Pheromone of the Banana Weevil (*Cosmopolites sordidus*).

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Cosmopolites sordidus, Germ. (banana weevil) is the most important world-wide insect pest of bananas. Several antennally active candidate pheromones have been detected in male-produced volatiles. The initial pheromone candidates to be identified are diastereoisomeric bicyclic ketals. Initial syntheses of mixtures of all possible stereoisomers did not provide sufficient material to establish laboratory or field activity (Beauhaire, J., et al.: Tet. Lett. 36, 1043, 1995). We report a convenient synthesis which provides large amounts of the candidate pheromone. We also report the relative attractiveness of diastereoisomers of the ketal candidate pheromone and development of an optimized pheromone-baited trap.

17-005

Pheromone-Based Management of Pests of Oil Palm

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Oil palm occupies approximately 300,000 Ha in Central and South America and 4,500,000 Ha in Southeast Asia. In the Americas the American palm weevil, *Rhynchophorus palmarum*, is a major threat to oil and coconut palm as a vector of red ring nematode. In 1991 a male-produced aggregation pheromone for this weevil was reported. Pheromone-based trapping of *R. palmarum* has been shown to lower red ring nematode infection rates by 80% over 1 year using 1 trap per 5 hectare. Pheromone-based trapping is currently used throughout Central and South America to reduce red ring disease in oil palm.

The coconut rhinoceros beetle, *Oryctes rhinoceros*, is the most important pest of young oil palm in Southeast Asia. A male-produced aggregation pheromone has recently (October, 1995) been reported for this pest. An operational pheromone-based trapping system has been developed which avoids the use of insecticides and is more cost effective than current population management which involves periodic inspection and insecticide treatment of young palms.

17-007

DEVELOPMENT OF SYSTEMS TO MONITOR CERATITIS CAPITATA AND SEVERAL ANASTREPHA SPECIES (DIPTERA: TEPHRITIDAE)

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Liquid protein-baited traps are currently used to capture males and females of several pest Tephritidae species. A synthetic food-based attractant and a painted cylindrical dry trap that protects the synthetic lures from the environment were developed as a replacement for liquid protein-baited traps. This trapping system was tested for capture of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and several *Anastrepha* species. The dry trap is constructed of acetate film and contains an internally-placed sticky insert. Field trials conducted in Central America and in several Mediterranean countries suggests that this newly designed trapping system affords a facile replacement of the liquid protein-baited traps currently in use. When used in conjunction with sterile insect release technology, the dry trap baited with food-based synthetic attractant often caught wild *C. capitata* in numbers equal to those caught by trimedlure-baited Jackson traps, but the dry trap caught many fewer sterile *C. capitata*.

17-008

THE IMPORTANCE OF THE INTERSPECIFIC COMPETITION BETWEEN TWO PARASITOID ON THE CONTROL OF BRUCHID POPULATIONS DURING STORAGE OF COWPEA IN BURKINA FASO.

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In Burkina Faso, two species of Coleoptera Bruchidae, *Callosobruchus maculatus* (F) and *Bruchidius atrolineatus* (Pic) cause important losses during the storage of cowpea (*Vigna unguiculata* Walp) in traditional granaries during the dry season. These bruchids colonized the cultures of cowpea at the end of the rainy season and reproduced on the maturing pods. The new generations of adults emerged in the granaries after harvest. Two generations of *B. atrolineatus* developed in the store but the adults of the second generation were in reproductive diapause. *C. maculatus* remained always sexually active, but 7 or 8 generations of beetles developed in the granaries from November to May. The population density remained low at the beginning of the storage and progressively increased from January when temperatures and then humidity became higher. Two larval parasitoids, *Dinarmus basalis* (Rond) and *Eupelmus vuilleti* (CRW) were present in the granaries but their influence on the bruchid population dynamic remained limited. During the storage, the density of *E. vuilleti* progressively increased whilst the *D. basalis* population density remained at a low level. The laboratory experiments demonstrated that the interspecific competition between the two parasitoid species explained this dominance of *E. vuilleti*. This competition limited the increase of the population of *D. basalis* which is the most efficient natural enemy of the bruchids in the stores.

17-010

IMPACT OF LARVAL FEEDING OF *SMICRONYX* SPP. (COL.: CURCULIONIDAE) ON SEED PRODUCTION OF *STRIGA HERMONTICA* (SCROPHULARIACEAE): AN HISTOLOGICAL STUDY

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Striga spp. (i.e witchweed) are parasitic weeds that cause severe reduction in yields of many crops grown in Africa especially in dry areas and on poor soils (Mboob,1989 ; Sauerborn, 1991 ; Parker & Riches, 1993). Among the 35 *Striga* species known to occur in Africa (Raynal-Roques, 1991), the most widely distributed is *Striga hermonthica* (Del.) Benth. (Scrophulariaceae). Our hypothesis was that *Striga* plant fitness should be reduced due to seed attack by developing larvae. Our objective was to assess, with histological techniques applied at the capsule level, the impact of *Smicronyx* larvae presence and feeding on *Striga* seed production. Doing so, we also obtained qualitative information on the histogenous and organogenous capacities of *S. hermonthica* ovary tissues during development of the *Smicronyx* larva. *Striga* plants were infested with *Smicronyx* in Burkina Faso. Galls were fixed in the field and brought back to the laboratory for histological work. Generally, only one larva lived per capsule. A single larva destroyed all seeds present in the capsule. *Smicronyx* curculios have therefore a potential as biocontrol agent of *Striga* spp.

17-009

HABITAT MANAGEMENT STRATEGIES FOR THE CONTROL OF INSECT PESTS IN THE TROPICS

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Studies on habitat management strategies, based on 'push-pull' approach included manipulation of intercropping, strip cropping, trap cropping, cover crop, relay cropping and no-tillage were undertaken to determine practices that suppress insect pests. No tillage with maize stubble in-situ resulted in high stem borer infestation and dead hearts in maize. Earlier planted trap crop provided some protection against stem borers. Broader row ratios in strips were effective in reducing stem borer and could be used without sacrificing the pest reduction potential of single alternate row intercropping. Planting maize into an already established cover crop, *Desmodium* sp. gave a dramatic reduction in stem borer incidence and damage. The underlying principles for the low pest incidence in habitat management are proffered. The principles led to the development and introduction of new cropping system alternative 'strip-relay cropping'. The indication are that the system is insect pest suppressing, increases land intensification and enhances economic profitability and could be an alternative cropping system for the humid tropics. Habitat manipulation has potential for use in environmentally sound pest management strategies.

17-011

APPLICATION OF *BACILLUS THURINGIENSIS* BERLINER ON DIFFERENT SORGHUM GENOTYPES SHOWING VARIABLE LEVELS OF RESISTANCE FOR CONTROL OF THE SPOTTED STEM BORER, *CHILO PARTELLUS* (SWINHOE) (LEPIDOPTERA: PYRALIDAE)

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Aqueous suspensions of a local *Bacillus thuringiensis* (Berliner) isolate identified as *B.t. kurstaki* has shown good potential for use in the control of the spotted stem borer, *Chilo partellus* (Swinhoe) when applied to 3 different sorghum genotypes under field conditions. The different sorghum genotypes comprised: a resistant (Hybrid-1) (Tx 623A X IS-1044), a tolerant (Gadam El-Hamam) (open pollinated variety), and a susceptible line (IS-18363) genotypes. This strain was locally produced on soybean/molasses medium and applied at the concentration of 3.7 X 10⁷ spores/ml. The effects of sorghum genotypes and *B. thuringiensis* were found to be separate. Susceptibility of *C. partellus* to *B. thuringiensis* was greater on the resistant than on the tolerant or susceptible genotypes. These results support the hypothesis that the susceptibility of insects to entomopathogens is inversely related to host plant suitability. In sorghum, the degradation by ultraviolet (uv) light is possibly not a crucial factor in influencing the field persistence of *B. thuringiensis*. This is due to the nature of the plant which provides shelter to the pathogen applied to the leaf whorl. The use of the uv-protectant Congo Red did not result in any improvement in the control of *C. partellus* compared to the application of *B. thuringiensis* alone. Infestation of sorghum plants with first-instar *C. partellus* up to 10 days after application of the pathogen to sorghum plants did not result in reduced grain yield compared to plants treated and infested on the same day.

17-012

MANAGING STEM BORERS IN SORGHUM AND PEARL MILLET

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Efforts in research to manage the major species of stem borers of sorghum (*Chilo partellus* in Africa and Asia, and *Busseola fusca* in Africa) and pearl millet (*Coniesta ignefusalis* in West Africa) have traditionally focused on the development of borer resistant cultivars. However, conventional breeding procedures are yet to produce acceptable genotypes. Recent shifts in focus are beginning to show good prospects.

In sorghum, increased farmer participatory inputs in the evaluation of improved sorghum cultivars and crop combinations have produced good results in Western Kenya. Recent studies both in Kenya and India show that parasitoids are more active in borer-damaged, moderately resistant sorghums and offer new prospects for interfacing biological control with host plant resistance. The potential impact of native ecosystems in this relationship is being studied. A modest effort has been initiated in India on marker-assisted selection procedures in pyramiding genes from diverse sources.

The pheromone-based management strategy for the millet stem borer has been successfully tested in several countries of Sahelian West Africa. This strategy involves a long-lasting pheromone dispenser impregnated with a synthetic blend and an in-expensive water-based trap designed from local materials. The wide-scale implementation of this strategy is expected to have a significant impact in reducing an estimated annual loss of \$91million caused by this pest.

17-014

TILLAGE EFFECTS ON WHITE GRUB LARVAE BY ANT PREDATORS IN TROPICAL MAIZE FIELDS.

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An eight year study of white grub infestation levels in no-till and conventional-till maize fields in Honduras indicated that white grub larvae became more abundant during the first two years of reduced tillage, but afterwards significantly declined in number.

It was also found that two ant species were significant predators of white grub eggs and early instar larvae. It appears that tillage may significantly affect ant predation and may influence the grub population fluctuations described.

17-013

SORGHUM INSECT PESTS AND THEIR MANAGEMENT IN THE TROPICS

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Although annual losses in sorghum grain production resulting from insect-induced crop damage are estimated at over \$1000 million in Africa and Asia and research on the major pest species: shoot fly (*Atherigona soccata*), stem borers (*Chilo partellus* and *Busseola fusca*), midge (*Contarinia sorghicola*) and head bugs (*Calocoris angustatus* and *Eurystylus oldii*) dates over 50 years, management strategies for these insects have been poorly focussed. The paper questions the scope for chemical insecticides in subsistence agriculture in India and sub-Saharan Africa, the non-adoption of recommended intercropping configurations and the limited impact in the breeding for pest resistant sorghums.

Pest management in the tropics is discussed in the context of indigenous farming systems and the need for a change in perception in the development of IPM programs. The prospects for applied biological control and the role of biotechnology in increasing levels of genetic resistance are examined. A call is made for increased attention to improved agronomic practices such as crop, soil and water management. Farmer-participatory inputs in the research process from the initial stages of constraint diagnosis, development and evaluation of options to the final stages of implementation are as crucial as the role of governments in adequate resource allocation, manpower development and better coordinated donor support.

17-015

THE IMPACT OF EGG PARASITISM ON BROWN PLANTHOPPER, *NILAPARVATA LUGENS* (STÅL) AND THE RELEVANCE TO PEST MANAGEMENT SYSTEMS FOR RICE IN ASIAM.F. Claridge, J.C. Morgan, A. Steenkiste, D. Damayante, D. Kertoseputro¹

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The importance of invertebrate predators of the various life history stages of *N. lugens* in providing high levels of natural field mortality is now well established in many parts of Asia. In a classic population study in the Philippines, Kenmore *et al.* (1985) demonstrated the importance of egg mortality due to a variety of uncertain causes, but including egg parasitism.

In Sri Lanka and Indonesia we have demonstrated a rich species diversity of egg parasitoids, including species of *Anagrus* (Hymenoptera: Mymaridae) and *Oligosita* (Hymenoptera: Trichogrammatidae). Levels of field mortality up to 80% have been observed. Parasitoid species tend to be more host specific than previously thought and show complex patterns of oviposition behaviour.

In Indonesia - 1. levels of parasitism have been monitored by using egg infested trap plants set out in the field; 2. field cage systems have been used to establish with certainty the relative effects of predators and parasitoids; 3. detailed attention has been given to the analysis of species composition of egg parasitoids and their relative abundances. Attempts have also been made to determine the effects on egg parasitism of habitat enrichment by encouraging the growth of particular weeds, including *Leersia hexandra*.

17-016

TERMITE DAMAGE ON MAIZE AND CONTROL
OPTIONS FOR SUBSISTENCE FARMERS.

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Termite, *Macrotermes Spp.* have been reported to cause considerable damage to maize in low rainfall areas of Eastern Kenya. Preventive and control methods have for a long time relied on persistent organochlorines. Due to environmental issues associated with these pesticides and their unavailability to subsistence farmers, experiments were conducted to investigate on other control alternative control methods using botanicals (Neem seed extract and wood ash) and manipulation of cropping systems that can reduce termite damage. Data collected included infestation and damage levels. Results indicated that maize beans intercrop reduced infestation, namely ground tunnelling, number of foraging termites and subsequent damage on maize. Wood ash and neem seed powder were also found to be effective in reducing infestation levels. However, termite infestation and damage on maize was found to be very much dependent on the amount of moisture in the soil. These alternative control methods might be cheaper for small scale farmers with no access to expensive pesticides.

17-018

IPM IN IRRIGATED RICE ENVIRONMENT : A SUCCESSFUL CASE
FROM BURKINA FASO.

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1. INERA, Laboratoire d'entomologie des céréales, Station de Farako-Bâ, Bobo-Dioulasso, BURKINA FASO. 2. Projet Vallée du Kou, Bobo-Dioulasso, BURKINA FASO. 3. ICIPE, Biomathematics Research Unit, Nairobi, KENYA. The efficiency and profitability of an IPM system based on phytosanitary survey and threshold interventions was evaluated in 30 small - scale farmer fields at the Vallée du Kou irrigated rice scheme for two consecutive crop seasons. There was a considerable advantage in cost, number of insecticide applications and yield of the IPM system as compared to an arbitrary or routine insecticidal application. The proposed method is considered to be environmentally friendly. A remarkable feature of the system is that farmers were involved in the set up and operation, and therefore likely to be sustainable. After the efficiency and profitability of the system being established, it was easily adopted by most of the farmers in the rice scheme.

KEY WORDS : Irrigated rice, stem borers, chemical control, phytosanitary survey, threshold interventions, IPM.

17-017

INTEGRATED PEST MANAGEMENT PROSPECTS FOR MAJOR
GRAMINACEOUS STEMBORERS IN SOUTHERN AFRICA

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In southern Africa maize, sorghum and sugarcane are grown on a large scale commercial basis. As such, these crops provide a large refuge for numerous insect species, a number of which are pests. Stem boring Lepidoptera are probably the most damaging in all three crops.

This paper will review published problems associated with chemical, cultural, biological and management pest control options against *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in maize and sorghum, and *Eldana saccharina* Walker (Lepidoptera: Pyralidae) in sugarcane. The prospects for integrating control options available to best control the different pests will be considered and examples of successful integrated pest management strategies against these stem borers in the various crops will be given.

17-019

PROSPECTS FOR THE CONTROL OF THE LARGER GRAIN BORER
PROSTEPHANUS TRUNCATUS (HORN.) IN AFRICA, USING
INTEGRATED PEST MANAGEMENT STRATEGIES.

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The Larger Grain Borer, *Prostephanus truncatus* (Horn.) an indigenous insect pest of dry maize in Central America, entered Africa and was first reported in Tanzania in the 1980s. Since then, it has spread to several countries including Kenya, Malawi, Zambia, Burundi, Rwanda in East Africa and Togo, Benin, Nigeria, Ghana, Burkina Faso, Niger and Guinea in West Africa, causing considerable damage to maize and dry cassava chips. In each of the affected countries, several methods are employed in an attempt to control this devastating insect pest. This paper examines prospects for the control of the Larger Grain Borer in its new home in Africa, using Integrated Pest Management Strategies.

17-020

ASSOCIATION AND SYNCHRONY OF *SMICRONYX GUINEANUS* VOSS, *SM. UMBRINUS* HUSTACHE (COLEOPTERA: CURCULIONIDAE) AND THE PARASITIC WEED *STRIGA HERMONTHICA* (DEL.) BENTH. (SCROPHULARIACEAE)

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Field experiments were conducted in 1992 and 1993 at Kaya, Burkina Faso, West Africa, in fields of sorghum (*Sorghum bicolor* (L.) Moench and pearl millet (*Pennisetum americanum* (L.) K. Schum. (syn. *P. typhoides* (Burm.) Stapf and Hubb.). *Striga hermonthica* was sampled weekly using a square meter metal frame. The sample size for each field was ten quadrats making a total of 10 m² in each field. Concurrently, adult populations of *Smicronyx guineanus* Voss and *Sm. umbrinus* Hustache were sampled using a Univac portable suction sampler to assess the synchrony of *Smicronyx* with *Striga*. Chi-square tests for independence of the populations of *Smicronyx* and *Striga* indicate a good synchrony of the active stages of the life-cycle of the weevils with the period of occurrence of the witchweed.

A 0.5 by 0.5 m metal frame was thrown ten times at random in each field and a Univac portable suction sampler was used to determine the degree of association between *Smicronyx* and *Striga*. There was a positive association between the weevil and the witchweed.

Key-words: *Smicronyx guineanus*, *Sm. umbrinus*, *Striga hermonthica*, identification, synchrony, association, galls, alternate hosts, biological control, Burkina Faso.

17-021

THE ECOLOGY OF *NEPHOTETTIX VIRESCENS* (DISTANT) (HEMIPTERA: CICADELLIDAE) IN RELATION TO ITS ROLE AS A VECTOR OF RICE TUNGRO VIRUSES
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The flight activity of *Nephotettix virescens* (Distant) in the Philippines was monitored by suction traps at 0.75 m and 1.5 m and an upwardly-directed segregating light trap at 4.0m and was closely associated with rice cropping patterns. Trap catches were largest towards the end of both wet and dry seasons, with lowest numbers recorded during fallow periods. The majority of individuals recorded in suction traps were caught around dusk with a smaller peak at dawn and larger catches were associated with increasing temperature. Results from sticky trap catches at canopy height indicated that adult *N. virescens*, particularly males, dispersed freely within rice plots at all crop growth stages.

Immigration of adult *N. virescens* into rice plots was greatest in wet seasons and was also high in late-planted dry season crops. Population density peaks occurred by 50-65 days after transplanting in wet seasons, but varied more widely in dry seasons. Tungro disease incidence was not directly related to leafhopper vector number. Rapid spread of tungro disease occurred in a late-planted dry season crop when peak adult numbers of *N. virescens* were only two per rice hill and nymphal populations were also low, but inoculum pressure was high. The results suggest that leafhopper vector numbers per se are not a sufficient indicator of tungro disease risk. In addition, the findings indicate that there are seasonal differences in inoculum pressure which may be taken into account in designing appropriate tungro management strategies.

17-023

BEMISIA TABACI AND AFRICAN CASSAVA MOSAIC DISEASE IN UGANDA AND THE CÔTE D'IVOIRE

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Detailed studies of the biology and ecology of the whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), the vector of African Cassava Mosaic Disease (ACMD), have recently been carried out in Uganda and the Côte d'Ivoire, providing an opportunity to compare two contrasting situations. In the Côte d'Ivoire, a relatively stable situation exists with ACMD infection consistently at c. 100% and yield-losses varying from 0-40%. Uganda, however, is currently experiencing an ACMD epidemic and transect surveys of the incidence of ACMD and *B. tabaci* abundance revealed a gradually moving 'front' of high ACMD incidence associated with increased *B. tabaci* numbers. Data on the phenology of *B. tabaci* populations and ACMD incidence in the contrasting agro-ecosystems will be presented and possible reasons for the observed differences will be discussed.

17-022

ECOLOGY OF SHARPSHOOTERS ASSOCIATED WITH *XYLELLA FASTIDIOSA* OF CITRUS IN BRAZIL

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Citrus variegated chlorosis is a new and destructive citrus disease caused by *Xylella fastidiosa*, first reported in São Paulo State, in 1987. Three sharpshooter leafhoppers (Hemiptera: Cicadellidae) frequently found in citrus trees, *Dilobopterus costalimai*, *Oncometopia fascialis* and *Acrogonia terminalis*, are likely field vectors of this bacteria. *D. costalimai* was already confirmed as an experimental vector. These xylem-feeding leafhoppers are widespread in southern Brazil, but are particularly abundant in northern São Paulo. Adult populations, as measured by yellow sticky cards, tend to increase during the summer, reach a peak in the fall (March-June), then decrease sharply in August and remain at low levels throughout the spring (September-November). Egg parasitism has been observed particularly in the spring. Spatial distribution of sharpshooters in the citrus orchards is usually aggregated. In the citrus trees, nymphs and adults show preference for young shoots facing the North. Observations of caged adults in the greenhouse indicated a higher flight activity around noon, when the temperature increases. Greenhouse studies showed that *D. costalimai* and *O. fascialis* can feed well and oviposit on various herbaceous weeds commonly found in citrus orchards. Some of these weeds are natural hosts of *X. fastidiosa* and might act as inoculum sources for primary spread of the disease.

17-024

BEHAVIOUR OF LEAFHOPPER VECTORS (*CICADULINA* spp.) WITHIN MAIZE AND ITS IMPORTANCE IN INCREASING MATING OPPORTUNITIES

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Studies in Uganda of the epidemiology of maize streak virus disease indicate that males and females of the vectors (*Cicadulina* spp.) differ in their behaviour within a maize crop. Up to 95% of *Cicadulina* caught in the upper canopy of maize are females and up to 90% caught close to the ground are males. The females show a preference for maize plants at 25-40 cm high (for var. Kawanda Composite A) and it is at this time that peak numbers of both sexes are found in maize plots. Results suggest that each maize plant can be visited by *Cicadulina* one to five times per day during the peak period. *C. mbila* and *C. storeyi* have been shown to communicate during mate seeking behaviour by vibrating on the plants they are resting on, the male instigating the communications. It is hypothesised that males accumulate in maize plots at the time that females are abundant, moving close to the ground where the vegetation is comparatively sparse, and testing maize stems in search of females sitting up in the whorls. It is suggested that weed-free maize plots provide good sites for mate seeking as the ability of males to locate females is enhanced. Most wild grasses, which are preferred hosts for feeding and oviposition, provide dense vegetation which is less suitable for mate seeking. This may explain why *Cicadulina* are, in general, only found in large numbers within or adjacent to crops. It should be possible to manipulate *Cicadulina* behaviour by making the crop less attractive for mating activities. Intercropping with plants which produce dense foliage near the ground appears to be one method of achieving a reduction in leafhopper mate seeking activity and thus reducing the incidence of maize streak virus disease.

17-026

BIOLOGY-ECOLOGY OF RICE HOJA BLANCA VIRUS VECTORS
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The delphacid *Tagosodes orizicolus* causes serious oviposition and feeding damage to rice cultivation in Latin America. It is also a vector for the severe rice hoja blanca virus disease (RHBV). Both rice and vector are hosts for the virus, which is transmitted both transovarially (80% efficiency) and horizontally from plant to insect (10% efficiency). The yeast-like endosymbionts essential for the insects' hormone metabolism, which are also transmitted transovarially, may be involved in either of the virus transmission modes. The characterisation of these endosymbionts from *T. orizicolus* and *T. cubanus* (vector of the related Echinochloa hoja blanca tenuivirus; EHBV) will be discussed. The insects' ability to acquire the virus from the plant is determined by a single recessive gene, hence the low horizontal transmission efficiency. In order to characterise this gene, and to analyse the population structure of the vector for epidemiological studies, the genetic variability of *T. orizicolus* was determined using RAPDs. These studies show little gene flow between isolated populations. So far, no PCR marker linked to the ability to acquire/transmit virus has yet been found. *T. orizicolus* is monophagous and one of its few alternative hosts is *Chloris radiata*, which may be a reservoir of insects or RHBV. Although closely related, *T. orizicolus* and *T. cubanus* do not feed on each others' main hosts (rice and *Echinochloa colona*), which may be the principal cause for the molecular divergence of RHBV and EHBV.

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17-025

DISPERSAL OF BEETLE AND LEAFHOPPER VECTORS TO AND FROM NATURAL AND AGROECOSYSTEMS IN THE NEOTROPICS

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Scientists from the US and Costa Rica collaborated in a cooperative project to survey plant virus vector taxa within tropical forests and neighboring agricultural settings in Costa Rica to determine if they differentially move between forests and adjoining croplands. The research emphasized vector taxa in the family Chrysomelidae (leaf beetles) (Coleoptera), and the Auchenorrhyncha (leafhoppers, planthoppers, treehoppers) (Homoptera) and their movements between natural and adjoining agricultural ecosystems. Targeted insect taxa were surveyed using large malaise traps positioned not only in both forested and pasture settings but also at the intersection of these two environments. The malaise traps used at the interface of forest and pasture were directional, i.e., they separated those insects flying into the forest from those flying into the pasture. Nearly 10,000 specimens within the target insect families were collected during the two-month rainy period of the first year of research. The data will be available to improve crop management and conservation biology efforts.

17-027

Comparison of intraspecific competition between latent and eruptive auchenorrhyncha (Homoptera) species on rice: *Recilia dorsalis* (Deltocephalidae) versus *Sogatella furcifera* (Delphacidae)
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A comparison of the effects of intraspecific competition between the white-backed planthopper (WBPH) *Sogatella furcifera* and the zigzag leafhopper (ZLH) *Recilia dorsalis*, as representatives of eruptive and latent auchenorrhyncha (Homoptera) species on rice, was carried out in laboratory. Crowding during the nymphal stage extended the duration of development of WBPH but not of ZLH. The nymphal survival rate of WBPH, sex ratio, preoviposition period and fecundity of both species were however not affected. The brachypterous rate of WBPH and nymphal survival of ZLH were significantly reduced under high nymphal density. Crowding in adult stage reduced significantly fecundity and longevity of both species. Fecundity and longevity was further reduced when combined with high density at nymphal stage. Age-specific life table analysis showed that the integrated effect of crowding on ZLH is stronger than on WBPH. The mechanism of eruptive and latent species' response to crowding during nymph and adult stages is not always clearly expressed as a significant difference in fecundity. It is better described as the variation in the intrinsic rate of increase (r_m). Therefore the construction of an age-specific life table is helpful, and perhaps necessary, to understand these effects. The main ecological characteristics of ZLH, as a latent species are its longer immature period, lower total fecundity and decreased and retarded rate of oviposition, hence the lower r_m , as compared with WBPH.

17-028

BENEFICIAL EFFECTS OF MAIZE MOLLICUTES ON THEIR NEOTROPICAL LEAFHOPPER VECTORS

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The neotropical leafhopper genera *Dalbulus* and *Balbulus* are comprised of 16 deltocephaline species that specialize on the native perennial gamagrasses (*Tripsacum*) and the teosintes (*Zea*). One leafhopper species, the corn leafhopper, *D. maidis*, has become a serious pest of the teosinte domesticate, maize, *Zea mays* subsp. *mays*. Only occasionally does the leafhopper cause economic loss via feeding damage, however, it is the most important vector of two plant pathogenic mollicutes, the corn stunt spiroplasma (CSS) and the maize bushy stunt phytoplasma (MBSP). The mollicutes propagate in and use leafhoppers as alternate hosts. Most leafhoppers in these genera are found on the gamagrasses which are immune to mollicute infection. Many of these species are capable of colonizing maize but when exposed to mollicute infected plants, adult life is significantly shortened and fecundity is markedly lowered. This may benefit *D. maidis* and a second maize specialist, *D. elimatus* whose life spans and fecundity are not affected by mollicute infection, from competition from their congeners in the maize habitat. Moreover, recent evidence suggests that maize mollicutes provide advantage to infected corn leafhoppers during the dry season in the neotropics when maize is not grown. Infected leafhoppers are more likely to survive temperature extremes and can live longer on alternate food hosts or on free water than noninfected leafhoppers. This mutualism will be discussed in relation to survival of mollicutes and their vectors and for management of these pests.

17-030

INVESTIGATIONS FOR THE NATURAL BIOLOGICAL CONTROL OF *ORYCTES CENTAURUS* (STERNB.) (COLEOPTERA: SCARABAEIDAE: DYNASTINAE) ON THE MAINLAND OF PAPUA NEW GUINEA.

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Oryctes centaurus mainly associated with sago palm (*Metroxylon sagu* Rottb.) has been also found breeding and attacking coconut palm (*Cocos nucifera* L.) on the mainland of Papua New Guinea. It may attack coconut palms in a similar manner to *O. rhinoceros*.

Volatile collections and electroantennography revealed that males produced a strongly EAD-active compound which is not produced by females. This male *O. centaurus* compound is also active on *O. rhinoceros* and *O. monoceros* antennae.

A "blue disease" has been found to occur in the natural breeding sites. First investigations revealed no causal agent. Electronic microscopic observations must be able to reveal whether a presumed pathogenic agent (virus) is enclosed in the spheroid vacuolated corpuscles already described in the past for *Oryctes* spp.

O. centaurus natural biological control knowledge may be useful to identify a possible control strategy for the most destructive coconut pests which occur on the Papua New Guinea islands, *Scapanes australis* and *Oryctes rhinoceros* (Coleoptera: Scarabaeidae: Dynastinae).

17-029

ASPECTS OF THE BIOLOGY OF THE COCONUT TERMITE *NEOTERMES RAINBOWI* (HILL) AND IMPLICATIONS FOR ITS MANAGEMENT

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Neotermes rainbowi has a restricted distribution on a number of coral atolls of the Cook Islands and Tuvalu in the South Pacific. The termites hollow out the trunks of coconut palms, rendering the palms vulnerable to windthrow, even by light winds. Young palms may be destroyed before reaching bearing age.

Alates, the king and queen, reach palms by air and establish a colony inside the bole. Alates are released from existing colonies in small numbers over extended periods of the year. On islands with no other competing (subterranean) termites, foragers of *N. rainbowi* can reach other palms via the soil or root connections. (Cook Islands). Where a species of *Nasutitermes* is present, colonies of the coconut termite are restricted to single palms (Tuvalu).

The coconut termite has a rather unique habit: it chews a system of channels and groves in the bark of the palm. The extent of bark markings roughly represents the occupied area of the trunk.

Once a palm snaps off, termites in the section of the palm left without the founding king and queen, will produce replacement reproductives (neotenics); the end result is two independent colonies. In *N. rainbowi* neotenics form within shorter periods and have a more balanced sex ratio than reported for other species of the genus. As palm stumps and broken trunks decay and the food supply becomes limited, development of most colony members will be channelled into alates. In response to pathogens, survivors can seal off contaminated areas of their tunnel system and wall off cadavers. Even small groups of survivors can re-establish themselves as fully functional colonies with the help of neotenics. Management options are (1) tree sanitation to reduce the release of colony-founding alates and (2) treatment of palms with the fungal pathogen *Metarhizium anisopliae*.

17-031

HEMIPTERA ON OIL PALM AND COCONUT, INCLUDING DISEASE VECTORS

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Many widely varying Hemiptera species are found on the leaves of palms grown in humid tropical zones.

Many of them are highly polyphagous, and only accidentally, generally once adult, end up on palm leaves, on which they can nevertheless feed. Others are more closely linked to palms and spend the whole of their development cycle on them, and lastly, some species seem to live on palms alone.

Most of the Hemiptera have no economic impact on their hosts, since population levels are rarely high enough for them to cause significant damage. However, some Heteroptera *Coreoidea* and *Coccoidea* species can be very damaging primary pests. Others, amongst the *Fulgoroidea*, *Cicadelloidea* and *Pentatomoidea*, transmit very serious diseases that have decimated oil palm and coconut plantations worldwide, for example coconut lethal yellowing in Africa and the Caribbean and oil palm and coconut trypanosome diseases in Latin America.

Lastly, some *Pentatomoidea* and *Reduviidae* species are auxiliary, attacking Lepidoptera caterpillars.

17-032

SYNTHETIC PHEROMONES USED SIMULTANEOUSLY TO TRAP TWO GENERA OF COCONUT BEETLE PESTS; *ORYCTES RHINOCEROS* L. AND *RHYNCHOPHORUS BILINEATUS* (Montr.) IN PAPUA NEW GUINEA AND THEIR INFECTION WITH PATHOGENIC ORGANISMS

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Synthetic pheromones originally researched by Oehlschlager at Simon Fraser University, Canada, were field tested in Papua New Guinea. Traps for the Asian rhinoceros beetle, *Oryctes rhinoceros* L. and the Black Palm Weevil, *Rhynchophorus bilineatus* (Montr.) were combined by placing the two different pheromones in the same trap. The addition of insect pathogens to some traps enabled continuous infection of live beetles which are returned to the field. The potential for utilising pheromone traps for disseminating insect pathogens is discussed.

17-033

INTEGRATED MANAGEMENT OF RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* F., IN DATE PALM PLANTATIONS OF THE KINGDOM OF SAUDI ARABIA

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The red palm weevil *Rhynchophorus ferrugineus* F. has become a serious pest of date palm in the Kingdom of Saudi Arabia, since its first report in 1987 in Al Qatif region of Eastern province. Subsequently the pest has spread to the regions of Al Hassa, besides 13 other areas in the Kingdom. For the management of the pest in these areas a comprehensive control program has been formulated and implemented with new strategies and methods developed and tested in the field. A systematic survey of the pest incidence in date palm farms was undertaken in all areas to identify the damaged plants. The palms detected in the early and medium stages of damage were treated by stem injections with an effective pesticide. The palms beyond recovery were either cut and burnt or treated with pesticide to kill all stages of the pest. To prevent attack, the palms were soaked regularly by an insecticide under low pressure.

Apart from these methods, pheromone lures baited with food were used for mass trapping as well as monitoring of the pest since 1994. For efficient pheromone trapping, lures with different release rates, trap design, density, location, food type, pesticide kind, food longevity etc. have been tested and standardized after field trials. In the areas of Al Qatif and Al Hassa pheromone based mass trapping has been successful in reducing the weevil populations and this was integrated with other methods for successful control of the pest. From an average infestation rate of 6.6% in 1993, in Al Qatif region the damage has been reduced to 4.0% in 1994 and 2.5% in 1995. In other regions also the results are satisfactory. By adopting various methods in an integrated way the infestations have been reduced over the past two years. The details of field trials and control program are presented in the paper.

17-034

INTEGRATED CONTROL OF *ORYCTES MONOCEROS* OL AND *RHYNCHOPHORUS PHOENICIS* (L.) IN NIGERIA

- PHEROMONE BASED MASS TRAPPING

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Three groups of 5 bucket traps containing *Oryctes monoceros* pheromone, enhanced with insecticide impregnated organic matter were set up on NIFOR date palm field at Dutse. A control set of 5 traps containing organic matter alone, without pheromone, was also set up. Traps were placed at 6 palms (52.8m) away from one another and examined once a week from April to May 1994. Pheromone traps enhanced with bits of sugar cane and cured cow dung or bits of palm fronds caught *Oryctes* while the traps without pheromone caught none. The difference was highly significant ($p < 0.05$). These traps lay out with little variation, and containing *Rhynchophorus phoenicis* pheromone were similarly set up on a replanted oil palm field in NIFOR, Benin City. Recoveries collated from February to May 1994 indicated that the *Rhynchophorus* pheromone was highly efficient ($p < 0.05$) in catching the adult insects. Pheromone based mass trapping will help immensely in protecting living palms from extensive destruction by these insects in Nigeria.

17-035

REDUCTION IN CANEGRUB POPULATION DENSITY WITH RETENTION OF CROP RESIDUES IN SUGARCANE (COLEOPTERA: SCARABAEIDAE).

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Sugarcane in Queensland, Australia, is harvested annually. An average of one plant crop and four ratoon crops is produced before replanting. Since 1940, sugarcane has been burnt before harvesting to remove trash and aid cutting. Modern machinery can harvest unburnt crops and retention of trash as a blanket on the soil surface is now widely practiced in North Queensland ratoon crops.

Lower population densities of greyback cane grub (*Dermolepida albohirtum*) (Waterhouse) were recorded in ratoon crops managed with green cane harvesting and trash blanketing (GCTB). A replicated trial at Tully Sugar Experiment Station compared GCTB with trash buried by cultivation, and with burnt trash. In the first ratoon, fewer grubs were found in GCTB (0.9 per stool) compared to buried trash (1.7 per stool) and burnt trash (1.6 per stool) (ANOVA $F_{(2,6)} = 4.6$; $P < 0.06$). Initial numbers of small cane grubs in the ratoon crop were similar in each treatment in the replicated trial at Tully. Rate of development and survival of cane grubs are lower under GCTB compared to burnt trash.

Four unreplicated trials compared GCTB with burnt trash on commercial farms, three in the Burdekin district in first ratoon blocks and one in Tully in fourth ratoon. Three sites had at least three times as many late-stage cane grubs per stool in crops with burnt trash compared to GCTB. One site gave 27% increase in tonnage of cane (103 cf. 131 tonnes/ha), and 30% higher sugar yield (13 cf. 17 tonnes/ha) in GCTB compared to burnt trash. The presence of grass weeds may have increased numbers of cane grubs in GCTB at the fourth site.

17-036

PROSPECTS OF USING PARASITIDS FOR TICK MANAGEMENT IN AFRICA.

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Although the existence of tick parasitoids has been known since the turn of the century their usability for tick control has not been fully investigated or exploited. Seven species of tick parasitoids have so far been described from the genus *Ixodiphagus*. In Africa tick parasitoids have been reported from South Africa, Uganda, Kenya, Nigeria, and Cote d'Ivoire.

Recent studies in Kenya have shown that tick parasitoids play a more important role in regulation of tick numbers in nature than previously thought.

In studies of *Ixodiphagus hookeri* a parasitoid of *Amblyomma variegatum* in Kenya, an experimental release of the parasitoid reduced the tick by over 80% within a period of one year. The experiment suggested that strategic releases of the parasitoid twice a year can keep the tick numbers down in semi-zero grazing type of husbandry.

This paper discusses the results of the pilot release study, analyses the status quo as regards studies on tick parasitoids in Africa and highlights what needs to be done if parasitoids are to be fully exploited for tick management in the continent.

17-037

THE ROLE OF WOMEN IN PEST MANAGEMENT: THE KENYAN PERSPECTIVE

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Despite food crop losses of about 50% due to pests, agriculture remains the main income earner for Kenya. Women manage 70% of the 4 million small scale farms in the country. They contribute in pest management by using cultural methods of farming such as inter-cropping, early planting, weed removal, crop rotation and burning of crop residues. This is however incidental rather than deliberate. They also use home made remedies for crop and post-harvest pests, as well for pests of animals such as ticks and tsetse.

Professionals such as veterinarians and those trained in agriculture contribute by teaching in universities, doing research and giving advice to farmers about pests. There are about 200 trained vets and a similar number in agriculture who have graduated from Kenyan Universities.

Involvement of women in pest management should include women groups. There are 23,000 such groups already registered in Kenya. Fundamental problems which women face, *inter alia*, lack of basic education, non participation in decision making, lack of professional training will have to be addressed if women are to participate in pest control more intelligently. There is need to inform and teach about IPM strategies, preservation of natural enemies of pests, and the benefits of cultural methods of farming as far as pests are concerned.

17-038

SPECIFICITY OF RESPONSE TO SEX PHEROMONES AMONG THE SWEET POTATO WEEVILS, *CYLAS PUNCTICOLLIS* BOHEMAN AND *C. BRUNNEUS* FABRICIUS (COLEOPTERA: APIONIDAE)

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The major components of the sex pheromones produced by females of the sweet potato weevils, *Cylas puncticollis* Boheman and *C. brunneus* Fabricius (Coleoptera: Apionidae), have been identified as decyl and dodecyl (*E*)-2-butenates respectively. Field tests in Uganda showed that the synthetic pheromones were highly attractive to males of the respective species, but, whereas synthetic lures for *C. puncticollis* were species-specific, those for *C. brunneus* also attracted significant numbers of *C. puncticollis* males. Females of both species attracted essentially only conspecific males, and further studies were carried out to determine how species-specificity of mating is ensured. In laboratory studies, no other pheromone components could be detected in female-produced volatiles from either species using gas chromatography linked to electroantennography (GC-EAG), and male weevils were found to give EAG responses to the pheromone of the other species. In the field, addition of even small amounts of the *C. puncticollis* pheromone to that of *C. brunneus* stopped attraction of *C. brunneus* males. However, investigation of the diurnal timing of captures at pheromone traps showed that peaks of male activity for the two species occur at quite different times during the night, ensuring species-specificity of mating. It is concluded that chemical identification of the pheromones is probably complete, and the consequences of these results for practical application of the synthetic pheromones are discussed.

17-039

LONG-RANGE SEX PHEROMONE OF THE LONGHORN BEETLE, *Migdolus fryanus* WESTWOOD: IDENTIFICATION AND APPLICATION

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The first long-range female-released sex pheromone for the family Cerambycidae is reported from a sugarcane pest in South and Central America, *Migdolus fryanus*. Two female-specific compounds were identified, namely, N-(2'S)-methylbutanoyl 2-methylbutylamine and N-formyl L-isoleucine methyl ester. Although the amide was very active in field tests, the amino acid derivative neither increased nor decreased trap catches by the amide. Recent experiments for the utilization of the synthetic sex pheromone in mating disruption will also be discussed.

17-040

WHY THE HARVESTER TERMITE, *HODOTERMES MOSSAMBICUS* (HAGEN), IS STILL A PEST IN SOUTH AFRICA.

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The harvester termite, *Hodotermes mossambicus*, has been considered a pest of natural pastures since 1934, because it competes with cattle for the available grazing, especially in drought years. However, its pest status needs to be re-evaluated as this termite has potential value in pasture ecosystems. Due to the continued pest status of the harvester termite, several techniques for chemical control have been developed. A thorough understanding of the biology and behaviour of the harvester termite should be used to improve the success of the methods used for control measures. Poisons need to target the final larval stage not the worker caste, as these larvae are responsible for feeding the colony. Research is continuing to investigate other non chemical means of control, such as fungicides or biological control, that can be used for termite population reduction.

17-042

DEVELOPING AND IMPLEMENTING INTEGRATED PEST MANAGEMENT OF CASSAVA GREEN MITES IN AFRICA

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The exotic cassava green mite, *Mononychellus tanajoa* (Bondar), is a major pest of cassava in Africa causing an estimated 30 to 50 percent reduction in yield. Increasingly, cassava farmers are demanding solutions. Ecological characterization of the pest has been used to target ecozones and to identify appropriate intervention technologies. These include classical biological control, host plant resistance and cultural practices. Two exotic phytoseiid mite predators have established, spread and significantly reduce pest populations in parts of the cassava belt. In addition, a virulent strain of an exotic fungal pathogen is being testing. Evidence of genetic resistance is prompting more cassava breeding programs to screen for mite damage symptoms. New varieties are now being developed, and their impact on the pest evaluated on-farm. Cultivars, soil fertility, time of planting, cropping system and time of harvest all influence the build-up of damaging pest populations and can be manipulated to reduce pest densities. Integrating these interventions into a comprehensive strategy remains a challenge.

17-041

FURTHER EVIDENCE FOR OVICIDAL AND LARVICIDAL ACTION OF NEEM, *AZADIRACHTA INDICA* A. JUSS, ON THE COWPEA POD BORER, *MARUCA TESTULALIS* GEYER

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Various neem preparations have been shown to interfere with feeding and/or growth and survival of larval stages of the legume pod borer, *Maruca testulalis* Geyer. However, nothing is known about the effect of neem on the eggs of this insect which are more vulnerable than the larval stage because they are fragile and laid in exposed locations on the cowpea plant. We therefore conducted laboratory studies to determine the effect of aqueous neem preparations on eggs, and included larvae for comparison.

High egg mortalities (30-71%) were obtained with 5, 10 and 20% (w/v) extracts. In addition to growth inhibition and high larval mortalities (28-94%) caused by the leaf extracts, seed extracts at the three doses tested completely prevented pupation.

If application of neem is timed to coincide with egg laying on cowpea by this pest, fewer individuals would be recruited into the larval stage. This should result in less crop damage and enhance larval control with neem or other interventions. Field studies are underway to test this hypothesis.

17-043

HOST-SIZE MEDIATED INTERACTION BETWEEN *GYRANUSOIDEA TEBYGI* AND *ANAGYRUS MANGICOLA* (HYMENOPTERA: ENCYRTIDAE), TWO PARASITOIDS INTRODUCED IN GABON AGAINST THE MANGO MEALYBUG

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Establishment, spread of *Anagyrus mangicola* Noyes and coexistence of the latter with the previously introduced parasitoid, *Gyranusoidea tebygi* Noyes, were assessed using emergence samples. Surveys indicated that *G. tebygi* which almost exclusively emerged from small hosts was present all over the surveyed area and predominated. In contrast, three years after introduction, *A. mangicola*, whose rates of emergence were similar among hosts sizes, was not recovered over a radius of 15 km from the release site where the host was scarcer. No evidence of differential hyperparasitism rates among survey areas with and those without *A. mangicola* was observed, indicating that speed of dispersal of this parasitoid was not affected by hyperparasitism.

Limited establishment and spread of *A. mangicola* to survey areas of highest to intermediate host densities could be explained by species differences in the number of offspring produced under various conditions of host sizes and densities.

17-044**EFFECT OF MIX-CROPPING ON PEANUT INSECT PEST POPULATIONS AND THEIR PREDATORS**

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Tests were initiated in 1990 and continued in 1991 at the University of Ouagadougou's experiment station at Gampela, Burkina Faso, to evaluate the effect of mix-cropping groundnut with sorghum or with pearl millet on insect pest damage and population densities. The impact of mix-cropping on predator population and groundnut pod yield was also examined. While population densities of the jassid, *Empoasca dolichi*, on groundnut were significantly reduced by mix-cropping. Both insect predator populations and pod yield were notably higher in diculture than in monoculture of groundnut.

Finally, our results show reduced foliage damage and already reported factors, such as improved light interception and soil moisture utilization by mix-cropped groundnut plants, to be complementary in enhancing pod yield in diculture of groundnut.

Key-words: mix-cropping, groundnut, sorghum, pearl millet, insect pest, predator arthropods, pod yield.

17-045

INSECT PREDATION BY SOME PONERINE ANTS AND
WEAVER SPIDERS IN COFFEE ORCHARDS IN
CHIAPAS, MEXICO

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Ants and spiders are among the most interesting predators for biological control in agroecosystems, due to their influence on pest-insects populations. To know the effect of these predators on insects populations, we sampled the prey caught by seven groups of predators, in coffee orchards in the Soconusco region of Chiapas, Mexico. The studied predators were two dominant ponerine ants: *Ectatomma ruidum* and *E. tuberculatum*, and five common weavers-spiders: *Cyclosa caroli*, *Gasteracantha cancriformis*, *Leucauge mandibulata/venusta*, *Anelosimus jucundus*, and Linyphiidae sp1 (probably *Lepthyphantes longispinosus*). We noted both the size of the webs and of the ants foraging areas, and their placement inside the orchards. Previously we sampled the coffee orchards to know (at family level) the kinds of arthropods living there, and their relative abundance. For the seven predators groups, the bulk of the prey (47.2% to 90.5%) belonged to four most abundant orders of insects: Hymenoptera, Diptera, Homoptera, and Coleoptera. In general, the frequency of relative predation on any prey, is proportional to their relative abundance. In spite of the differences in predation efficiency found among the studied predators, their activity did not result in a competition, but in a synergy. On the whole, their predation ranges did not overlap: the soil for *E. ruidum*, the coffee plants for the spiders (each web type placed on certain zone inside the foliage, or between coffee plants), and the shadow trees and branches of the coffee plants for *E. tuberculatum*. So the integration of the predation ranges of these seven groups of predators, makes them a very important assemblage of biological control agents for the coffee orchards in this neotropical region.

17-047

TETHERED FLIGHT PERFORMANCE OF LEAFHOPPER
VECTORS, *NEPHOTETTIX VIRESCENS* (DISTANT), *N.*
NIGROPICUS (STÅL) AND *RECILIA DORSALIS*
(MOTSCHULSKY) (HEMIPTERA: CICADELLIDAE) OF RICE
TUNGRO DISEASE.

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A collaborative project between the Natural Resources Institute and the International Rice Research Institute (Philippines) is studying the epidemiology of rice tungro disease (RTD) and ecology of its leafhopper vectors. The flight performance of the main tropical vectors of RTD, *Nephotettix virescens* (Distant), *N. nigropictus* (Stål) and *Recilia dorsalis* (Motschulsky) has been studied. The effects of various biotic factors, including host plant age and variety and rearing on RTD virus infected plants, on the flight performance of leafhoppers tethered to flight mills has been investigated. It has been shown that under certain conditions a proportion of the adult *N. virescens* population have the potential to make flights of maximum duration similar to those recorded from individuals of *Nilaparvata lugens* Stål from migrant populations. When field-caught *N. virescens* produced progeny on late season (mature) rice plants a higher proportion of the resulting adults could be induced to fly and a significant number undertook very long flights. When reared on *Nephotettix*-resistant rice plants the small number that survived to adults were also 'good' fliers. Insects reared through one generation on RTD-infected plants were less willing to fly whereas those tested after a 24 hour access period to RTD-infected plants were more flight-willing compared to controls (maintained on and moved to uninfected plants of the same age and variety). The possible relevance of these findings to the spread of RTD is described and related to possible integrated pest management interventions for the control of the disease.

17-046

NEW OPTIONS FOR THE INTEGRATED MANAGEMENT OF
SORGHUM HEAD BUG *EURYSTYLUS OLDI*
(HETEROPTERA:MIRIDAE) IN WEST AFRICA

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Host plant resistance (HPR) has so far been the mainstay of control strategies of *Eurystylus oldi* Poppius (Heteroptera: Miridae), a major panicle feeding pest of sorghum in West Africa. However, research work carried out in Mali in 1994-95 provided new options for the management of this insect. Several natural enemies, particularly assassin bugs (Heteroptera: Reduviidae) were identified. Castor (*Ricinus communis* L.) was identified as an alternate host for *E. oldi* during the dry season, providing an explanation of the population carry-over of this pest. The protection of sorghum panicles with extracts of physic nut (*Jatropha curcas* L.) translated into a reduction of infestation and damage by *E. oldi*. These promising results are discussed in view of the integrated bio-intensive management of *E. oldi* in West Africa based on HPR and other components such as: conservation of natural enemies; interruption of infestation cycle through control of alternate hosts; and use of locally available plant-derived pesticides.

17-048

DATE PALM TREES DAMAGED BY SOME INSECTS
IN THE STATE OF QATAR

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The date palm is an important plant which provides a primary article of food and commerce in the great desert areas extending from western north Africa to India. The total number of Date-palm trees in the world is about 88.50 millions, More than about 70% of this number is found in Arab countries. The Arab world produces more than 80% of the world production of the Dates. In the state of Qatar more than 500.000 Date-palm trees are found. Many insects attack, leaves fruit and trunks. Such as *Porlоторia blanchardi* Targ., *Asterolecanium phoenici* Rao. and different Spp. of termites. The most dangerous insect-pests are the Indian palm weevils {Red palm weevils} *Rhynchophorus ferrugineus*, palm stem bores *Pseudophilus testaceus* Graham. and fruit stalk bores *orytes elegans* preL. Biology, estimation of damage as well as methods of control are under investigation.

17-049

ARTIFICIAL REARING OF THE ASIATIC PALM WEEVIL, *RHABDOSCELUS LINEATICOLLIS* HELLER (COLEOPTERA: RHYNCHOPHORIDAE).

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The Asiatic palm weevil, *Rhabdoscelus lineaticollis*, has been known as a pest of palm. But this weevil was collected from sugarcane stalks in Gushikawa City, Okinawa Island in 1993. Since then, this species has become a serious pest in sugarcane field on a few islands of Okinawa. However, a few studies for ecology and life history of the weevil had been conducted.

In this study, an artificial rearing method of *Rhabdoscelus lineaticollis* using an established diet for silkworm (INSECTA-LF) was developed. The method has made possible successful rearing from egg to adult of the weevil. The weevil had 5~7 larval instars and its prepupal stage, and the mean egg, larval, prepupal, and pupal periods were 4.75 ± 0.44 , 29.33 ± 8.88 , 18.97 ± 5.68 and 9.17 ± 0.94 days, respectively, at 28°C under the artificial rearing condition.

17-050

DEVELOPMENT OF PHEROMONE TRAPS FOR CONTROL AND MONITORING OF SWEET POTATO WEEVILS, *CYLAS PUNCTICOLLIS* BOHEMAN AND *C. BRUNNEUS* FABRICIUS (COLEOPTERA: APIONIDAE) IN UGANDA

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Following chemical identification of the sex pheromones of the sweet potato weevils *Cylas puncticollis* Boheman and *C. brunneus* Fabricius (Coleoptera: Apionidae), work is in progress in Uganda to develop pheromone traps for use in control and monitoring programmes. Experiments have been conducted to optimise several parameters associated with the traps.

Of six designs evaluated, a 5 litre plastic jerry-can trap proved effective and was adopted as the simplest and most robust design. A sticky-disc trap, originally designed for catching armyworm moths in Malawi, and a skirted funnel design modelled on that used in the USA with *C. formicarius*, successfully caught weevils but were impractical to use and expensive to make. With the jerry-can trap the effects of colour and height above ground were investigated. Comparisons of lure dispenser type and different pheromone loadings were also carried out, and lures were developed for both species which showed no loss of attractiveness during up to eight weeks field exposure.

These traps and lures have potential for use in the control and monitoring of sweet potato weevils in Uganda, and results of experiments to determine trap sampling range and to evaluate the effectiveness of control by mass-trapping will be presented.

17-051

INFLUENCE OF AGE AND DIET ON MATING SUCCESS OF *PLUTELLA XYLOSTELLA* (L.)

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Plutella xylostella raised on *Brassica juncea* and artificial diet were used in reciprocal and control crosses to study mating success at various ages (1-5 days) of the adult moths. Positive results were indicated by transfer of the spermatophore to female individuals within 48 hours. Age has a significant effect on spermatophore transfer, with greatest mating success for 5 day old males and females. Age also has significant effect on number of larvae produced, with 1 day old females and 2 day old males producing most progeny. However there is no significant difference between number of eggs laid by females of different ages. The cross of males raised on *Brassica* with females raised on artificial diet gave more progeny as compared to the reciprocal cross and the controls. These findings may need to be considered in sterile insect technique programmes for control of *Plutella xylostella*.

17-052

DUNG BEETLES (COLEOPTERA: SCARABAEOIDEA) FEEDING ON DIPLOPOD CARCASSES

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Among the enormous number of dung beetle species in West Africa some feed on diplopod carcasses instead of dung. In Ivory Coast CAMBEFORT (1984) found 5 species of Scarabaeidae more or less specialized on this resource.

In the Parc National de la Comoé in the Guinea savanna region of this country we found 50 species ($n = 1151$) of Scarabaeoidea on dead diplopods (mostly Spirostreptidae) during the wet season in the gallery forest. Some of them are generalists feeding on dung and/or different carcasses (*Anachalcos*, *Onitis*, *Trox*). Others are specialists, among them the dominant species (75,6 % of all specimens), *Onthophagus latigibber* D'ORB.

References

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17-053

PRELIMINARY STUDY ON NECROCENOSSES IN THE GUINEA SAVANNA IN THE IVORY COAST (COLEOPTERA)

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In the Afrotropical region necrocenoses are dominated by Scarabaeoidea (Trogidae, Hybosoridae, Scarabaeidae). The role of carrion beetles (Silphidae) is negligible.

During the wet season (July 1995) in the Parc National de la Comoé in northern Ivory Coast (in both Guinea savanna and gallery forest), we found among 2744 beetles only 16 Silphidae, but 1826 Scarabaeoidea on fresh goat skins. Skins deposited in the gallery forest are less attractive to beetles than those placed in the savanna. During the first days, Gymnopleurini are the dominant diurnal and Onthophagini the dominant nocturnal group. After the fourth day Onthophagini become dominant at day and Trogidae at night.

First countings during other months show that in the beginning of the wet season (April/May) the number of beetles per carrion is much higher than in July. Especially the diurnal *Gymnopleurus puncticollis* (Scarabaeidae) and the nocturnal *Phaeochrous* (Hybosoridae) occur at very high numbers.

During the dry season (December), the number of beetles per carrion is reduced; the dominating scarab guild are the Onthophagini; Trogidae are rare, *Phaeochrous* seems to be absent.

17-055

CONTROL OF THE CARAMBOLA FRUIT FLY, *Bactrocera carambolae* (DIPTERA : TEPHRITIDAE) BY MALE ANNIHILATION METHOD AND WRAPPING

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The Carambola fruit fly, *Bactrocera carambolae* Drew & Hancock, is one of the major pests of star-fruit or carambola, *Averrhoa carambola*. Control study of this fly was carried out from March 1994 to January 1995 at a commercial carambola fruit orchard in Cirebon, West Java, Indonesia. Two type of trap (Delta and Round Trap) with four different solution of Methyl Eugenol (ME) as an attractant were applied at the beginning of the florescence. One month after this period, conventional preventive method was applied by wrapping individual fruit with a plastic transparent bag. The results showed that Round Trap with pure ME attracted the most number of flies. The number of flies caught by ME diluted by two times of commercial oil, were not significantly different with that caught by pure ME with both in Round Trap and Delta Trap. During this study, the population number of carambola fruit flies started to increase in May, at the beginning of florescence, then reached the peak in July to August 1994 when the fruit ripened. The population was then decreased at the end. This pattern was the same to the following season (September, 1994 - January, 1995). From fruit production data, it is indicate that application of the male annihilation method and wrapping using ME reduce the infestation of the fruit flies up to one fourth, which is from 25% to 6%.

17-054

EDGE EFFECTS ON AMBROSIA BEETLE COMMUNITIES IN A LOWLAND TROPICAL RAIN FOREST, BORDERING OIL-PALM PLANTATIONS, IN PENINSULAR MALAYSIA

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When the aim of forest conservation is to preserve interior forest conditions as being representative of the original forest, it is essential to understand how they may be influenced by edge or border effects. We investigated edge effects on ambrosia beetle communities in Pasoh Forest Reserve (2,450 ha), a lowland rain forest in Peninsular Malaysia, which largely borders oil-palm plantations established in the 1970's.

Species richness and composition of the ambrosia beetles (Scolytidae: Xyleborini) sampled with ethanol traps was similar along a gradient from the core area to the boundary of the forest. However, a polyphagous species, *Xylosandrus crassiusculus*, consistently increased in numbers from the core to the forest edge, being super-dominant in the boundary. It was as abundant in the surrounding oil-palm plantation as in the forest boundary. The results strongly suggest that there is a large flux of the cosmopolitic species from the oil-palm plantations deep into the forest. The population may also be, to a lesser extent, enhanced by forest disturbance in the margin of the reserve. It is suggested that interior forest conditions are affected by the influx of the ambrosia beetle since, for example, there is a possibility the insect may carry microorganisms originating from the surrounding, disturbed habitat. A wide buffer zone, at least 2 km in width, will be necessary to efface such external influence.

17-056

BIOLOGY OF PULSE BEETLE *CALLOSOBRUCHUS CHINENSIS* (L.) ON GREENGRAM, *VIGNA RADIATA* (L.) WILCZEK UNDER LABORATORY CONDITIONS

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Consequent upon the establishment of 1.8% field infested greengram pods and emergence of 6 males and 5 females beetles from these pods in the laboratory, observations on biology of pulse beetle, *C. chinensis* were recorded on greengram, *V. radiata* by releasing freshly emerged one pair of beetle in glass vial enclosing four dry and ripened greengram pods. There were 25 replications. Preoviposition, oviposition and post-oviposition periods of pulse beetle were found to be on an average 10.8 (8-11 hrs.), 5.6 (Range 4-8 days) and 1.5 (Range 1-3 days) respectively. The longevity period of female of pulse beetle was 6.6 days (Range 4-8 days) as compared to 6.3 days (Range 5-9 days) of male. A single female of pulse beetle laid on average of 44.5 eggs (Range 28-59 eggs) and the maximum egg laying was recorded on the first day of oviposition after that egg laying went on decreasing till the last day of oviposition. Incubation and total development period from egg to adult emergence were observed to be with an average of 6.1 (Range 6-7) and 28.3 days (Range 26-31 days), respectively. Out of 1112 eggs, a mean of 12.3% adults (Range 6.8-18.1% adults) emerged under laboratory conditions.

17-057

ADELENCYRTUS MODERATUS (HOWARD) (HYME-NOPTERA: ENCARTIDAE) IN THE CONTROL OF *ASPIDIELLA HARTII* (COCKERELL) (HOMOPTERA: DIASPIDIDAE) ON *DIOSCOREA* CFR. *ALATA* L. IN WEST AFRICA

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A major infestation of yam scale, *Aspidiella hartii* (Cockerell), was found on tubers of *Dioscorea* cfr. *alata* L., imported to Italy from Ghana for food use and intercepted by the Servizio Fitosanitario Regionale.

On random samples of parings of infested tubers, measuring about 5 cm², representative of the whole batch imported and isolated in a laboratory, on 2nd-instar larvae and females of *A. hartii* (number of scales present averaged 24,11/cm² tuber) there was found *Adelencyrtus moderatus* (Howard) parasitization ranging from 37,04% to 86,36%, with an average of 56,57%. In laboratory conditions, it was observed that there is very high emergence, amounting to 96,62%.

From the samples there also collected some individuals of *Azotus* sp. (Hymenoptera: Aphelinidae). It remains to be verified whether this was a case of primary parasite activity on yam scale, or hyperparasite activity on *A. moderatus*.

17-058

THE ROLE OF SEMIOCHEMICALS IN DIFFERENTIAL INFESTATION OF CALLOSOBRUCHUS MACULATUS IN WILD AND CULTIVATED PULSES

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Primary metabolites comprising of protein, carbohydrates, lipids and free amino acids, as well as secondary metabolites such as total phenols and ortho-dihydroxy phenols were analysed in wild and cultivated pulses. Trypsin inhibitor level was also quantified. Studies on development of *Callosobruchus maculatus* on wild and cultivated pulse seeds were made. The content of primary metabolites in the cultivars was higher than in the wild relatives. The secondary metabolites of the cultivars did not differ much from the wild relatives. The trypsin inhibitor levels were quite varied within the wild pulse populations both at intra and interspecific levels. A drastic reduction in the egg laying capacity of *Callosobruchus maculatus* was observed in the wild pulses. Our biochemical analyses suggest that the wild pulses contain compounds which inhibit egg laying.

17-059

BRUCHID ECOTYPES IN NIGERIA - THE CASE OF *CALLOSOBRUCHUS MACULATUS* FAB ON COWPEA, *VIGNA UNGUICULATA* (L.) WALP.

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Variation in insect response to resistant crop varieties is a common phenomenon. This is sometimes the result of ecological exclusion or other barriers that restrict gene flow between individuals or regions, and appears to be true of the cowpea storage bruchid, *Callosobruchus maculatus* Fab. We studied oviposition, growth and development of six populations of *C. maculatus*, designated simply as IT, PH, UM, MD-1, MD-2, MD-3, from different locations in Nigeria on the resistant cowpea cultivar, TVu 2027 and four cultivars of African yam bean (AYB) (*Stenostylis sternocarpa*) obtained from the same eastern Nigeria location as one of the bruchid populations, and on Ife Brown cowpea cultivar (susceptible). F1 progenies obtained by cross-mating individuals from different populations were similarly tested on the control cultivars.

Oviposition was variable, and differences were not significant. The lowest egg count was from the UM population. The IT and PH populations had higher adult emergence and shorter developmental times on TVu 2027 than did the other populations. This was not surprising for the IT population that, unlike the PH population, had previous experience with TVu 2027. All F1 populations with IT female parents also had better emergence and developmental period than their reciprocal crosses, or those involving other populations but this varied with the virulence of the male parent. The UM population was one of those most sensitive to TVu 2027 (average <10% emergence), but also seemed better adapted to the AYB cultivars (66% emergence compared to 71.9% for the susceptible control). All others ranged in emergence from 1.4-22.8%. Total development time was correspondingly shorter in the UM population. These results suggest that there is only one species involved. However, there appears to be distinct ecotypes of this species in Nigeria. This has far-reaching implications for the development and deployment of cowpea cultivars that have resistance to only one of the ecotypes.

17-060

Anastrepha fraterculus (Diptera: Tephritidae) dispersal in Brazilian apple growing area

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Apple growing area expanded from 137 ha in 1970 to more than 30,000 ha in 1995. *Anastrepha fraterculus* is a major pest and hosts in native vegetation areas adjacent to commercial orchards are responsible for population growth.

A feral population was released in order to study *A. fraterculus* dispersive behavior. Marked adults were maintained in lab conditions up to 10 days with water and food *ad libitum*. McPhail traps containing grape juice 25% were used: 15 in a 0.5 ha native vegetation area, 24 in an open grass field, and 48 in a 7 ha commercial orchard. Adults were released in the edge of the native vegetation area.

Out of 2,154 released flies, 7.1% were recaptured from the 2nd to the 20th day after release. Four, 12 and 17 days after release, 27.6, 88.0 and 98.4% of recaptures had occurred. Out of 8 flies (7 females: 1 male) recaptured in the orchard, 6 were recaptured in the edge (580 m from release point), one at 670 m and one at 700 m. These flies may have been helped by the wind. In the orchard, recaptures occurred 3 and 4 days after release. An insecticide cover spray may have affected results. In the native vegetation area, 144 marked flies were recaptured and behaved similarly to wild flies. No flies were recaptured in the grass field.

Results suggest that *A. fraterculus* dispersive behavior from native vegetation areas into orchards explains the large attack intensity in orchard edges.

17-061

Status of 'Golden Delicious' apples as *Anastrepha fraterculus* (Diptera: Tephritidae) hosts in BrazilR.L. Sugayama¹, A. Kovaleski^{1,2}, E.S. Branco³ & A. Malavasi¹¹Dept. Biologia, Inst. Biociências, USP, São Paulo, ²EMBRAPA/CNPV, Vacaria, ³ESALQ/USP, Piracicaba, Brazil.

Behavioral and physiological constraints can limit host range expansion in phytophagous insects. Apples were introduced in Southern Brazil in the early 70's and *Anastrepha fraterculus* (Wied.) became an important pest. It is a neotropical and polyphagous fruit fly and its major hosts are from the family Myrtaceae.

Behavioral observations in an unsprayed orchard showed that females oviposited intensely in apples. They were observed to bore up to 7 times in 5 min. It should be considered that there is a strong behavioral pressure to colonization of apples.

In this orchard, infestation level was 0.22 pupae/fruit in 1995/96, although all fruits were severely attacked (> 20 punctures/fruit), suggesting a high mortality in egg and larval stages. Dissection of immature fruits showed a large proportion of unhatched eggs. Larval development took up to 90 days in natural condition. Wild pupae obtained from apples and primary hosts did not differ in size. When compared to adults obtained in primary hosts, those from apples had lower survivorship leading to lower reproductive rates but do not affecting intrinsic rate of increase (*r*). This reflects the colonizing characteristic of the species. Since adults from apples are as aggressive as those from primary hosts, physiological constraints should also be considered overcome.

'Golden Delicious' apples can be considered satisfactory *A. fraterculus* hosts. Results indicated that the species is adapted to utilization of heterogeneous resources and that, in the absence of primary hosts, alternative hosts can support population growth.

FAPESP, EMBRAPA

17-063

TRICHOMES AS A PLANT TRAIT INFLUENCING HERBIVORE DISTRIBUTION AND ABUNDANCE

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The study was carried out during 1993-95 at three field sites within the cerrado *sensu stricto* near Brasília in central Brazil. We compared three *Byrsonima* (Malpighiaceae) host plant species with different degrees of leaf pubescence as larval food for some specific larvae species. The plant species were: *B. verbascifolia* (denser pubescence), *B. crassa* (less dense), and *B. coccolobifolia* (glabrous). A survey conducted on 5530 individuals of *Byrsonima* spp showed that, of the 53 caterpillar species found, approximately 23% belonged to Oecophoridae, with *Cerconota achatina*, *Gonioterma exquisita*, and *G. indecora* the most representative moths. Other common species were a gelechiid (*Anacampsis* sp), a hesperiid (*Chiomara punctum*), and two chrysomelids (*Chlamisus* spp) (Coleoptera). All these species were apparently specialists on *Byrsonima*. By comparing the frequency and distribution of insects on each host plant species, we noted the species *G. exquisita* and *Anacampsis* sp were never encountered (*n* = 1956) on the glabrous host *B. coccolobifolia*, which was preferred by *C. achatina*, and *C. punctum*. These results reflect not only the feeding preference but also may be related to larvae sheltering strategy. The first two species, were always found using the leaves trichomes for their larvae shelter while *C. achatina* and *C. punctum* that use a glabrous host, building their shelter by folding a piece of leaf. Another differential response to trichome density was also found for the beetles *Chlamisus* which cover their larval shelter with several layers of leaf hairs. All these strategies may be very important in the cerrado area which has a marked dry season from April to September. *G. indecora* feeds on all three plant species, but does not fold leaves or use the trichomes; instead, it ties leaves with silk around shelter. This study provides preliminary information indicating that plant pubescence may be related to herbivorous sheltering strategies and hence may influence their distribution and abundance among host plant species.

17-062

PRESENT STATE OF THE LIRIOMYZA LEAFMINER (DIPTERA: AGROMYZIDAE) PROBLEM IN CHINA

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For the past 3 years China has undergone wide-ranging outbreaks of the *Liriomyza* leafminers. The leafminers that severely damaged several ten species of vegetable and ornamental crops in China are obviously polyphagous species. There are leafminer problems and outbreaks in at least fifteen provinces of China. The leafminers mainly destroy the vegetable crops on the opening fields in South, and in greenhouse in North of China. Compared to other regions, for example, North and South America, Europe, where *Liriomyza* leafminers have caused damage to vegetable and ornamental crops since the end of the World War II, the *Liriomyza* problem in China is relatively recent. Although international trades play an important role in leafminer problems, the major factors that have originated this problem in China are related to the increase of vegetable planting area, wide use of pesticide, introduction of new crops, and to some extent the lack of monitoring and vigilance. The suppression of the leafminers relies largely on the chemical pesticides in China, because some chemicals can provide satisfactory control. However, the resistance of the leafminers to pesticides still maintains unknown. The studies on alternative control methods, ecology and biological control of the leafminers are being carried out in China in order to undertake a long term project to establish an integrated leafminer control program. Prospective control strategies are discussed, including cultural management, introduction of natural enemies, and effective utilization of appropriate chemical measures based on phenology forecast and growing stages of the plants.

17-064

BIOLOGICAL STUDIES OF *Leptoglossus chilensis concaviusculus* BERG, 1892 (HEMIPTERA: COREIDAE) ON ITS NATURAL HOST PLANT, *Schinus terebinthifolius* RADDI (ANACARDIACEAE) UNDER CONTROLLED LABORATORY CONDITIONS.

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The coreid bug *Leptoglossus chilensis concaviusculus* frequently occurs in pepper trees, *Schinus terebinthifolius*, which is widely used as an ornamental plant in Brazil. The genus *Leptoglossus* is predominantly neotropical and contains some economically important species attacking fruits and seeds of many useful plants. The present study aimed to investigate the biology of this coreid bug on its natural host under laboratory conditions. The study was carried out at the insectary of the Zoology Department, UNICAMP during 1992-1995, at $25 \pm 2^\circ\text{C}$, $60 \pm 10\%$ R.H. and 14 h. photofase. The incubation period of eggs lasted from 9 to 15 days, with an average of 12.27 ± 0.026 days. Effect of aggregation on the nymphal and adult developments was studied. The entire nymphal stage consists of five instars, which lasted 35.45 ± 0.15 and 33.70 ± 0.38 days, for aggregated and individualized nymphs, respectively. The average longevity of mated adults, originated from aggregated nymphs, was 60.53 ± 5.23 days, while those originated from individualized ones lasted an average of 95.92 ± 7.83 days. The females suffering the aggregation effect deposited an average of 43.5 ± 20.9 , with a maximum of 228 and a minimum of 10 eggs/female. However, adults without such a stress deposited an average of 68.33 ± 28.96 , with a maximum of 356 and a minimum of only one eggs/female. Other aspects concerning developmental and reproductive activities were also investigated. Significant differences confirmed the stress effect of aggregation under laboratory conditions.

17-065

REPRODUCTION AND DEVELOPMENT OF *Camptischium clavipes* (FABRICIUS, 1803) (HEMIPTERA, COREIDAE) UNDER LABORATORY CONDITIONS

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Camptischium clavipes, a coreid bug, attacks leaves, buds and fruits of some Euphorbiaceae, Leguminosae, Solanaceae and Cucurbitaceae. Some reproductive and developmental aspects were investigated in the present work. Nymphs and adults were fed on *Ricinus communis* L. (Euphorbiaceae) leaves and sugar solution (10%) and the observations were realized under laboratory conditions of $25 \pm 2^\circ\text{C}$, $60 \pm 10\%$ of R.H. and 14 hours of photophase.

Adult longevity ($n=30$) was averaged 94.15 ± 4.83 days, reaching a maximum of 126 and a minimum of 41 days. No significant differences were observed between mated males and females. The female deposits an average of 304.46 ± 46.18 eggs during the oviposition period, with a maximum of 650 and a minimum of 55 eggs. The eggs are deposited in clutches, which averaged 15.40 ± 1.94 per female, with a maximum of 27 and a minimum of 4 clutches. Other approaches concerning reproductive activities were investigated.

The incubation period of the egg stage ($n = 3006$) reached an average of 12.54 ± 0.01 days with a maximum of 16 and a minimum of 8 days. Morphological descriptions and measurements as well as the viability of this stage were also obtained. The nymphal stage, consisting of 5 instars, lasted an average of 50.94 ± 2.4 days with a maximum of 71 and a minimum of 38 days. The sex ratio was calculated as 0.47.

17-067

IDENTIFICATION OF KEY-FACTORS OF GROUNDNUT MORTALITY IN THE FIELD

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Experimental studies were conducted in 1990 and 1991 at the Research Stations of Gampela and Matourkou, Burkina Faso, to assess plant, flower and peg pod mortality of ground-nut grown in the field, to identify main mortality factors and to determine the impact of various mortalities on potential yield loss. Evaluation techniques used included mortality tables and chemical exclusion. Our results show that plant mortality occurs mostly during plant growth stages preceding flowering. Soil fungi, *Aspergillus* sp., *Sclerotium* sp. and *Fusarium* sp. were the main factors involved in ground-nut mortality at these stages. In contrast, pest arthropods, i. e., millipedes, termites, thrips and blister beetles damage ground-nut mainly during the reproductive stages.

It appears from the present study that seed treatment with appropriate fungicide and insecticide apply from ground-nut flowering stage could prevent yield loss up to 19-20%.

Key-words: *Arachis hypogaea*, Ground-nut, mortality tables, pesticides exclusion, mortality factors, soil fungi, pest arthropods, potential yield loss, phytopathology.

17-066

SURVEY OF ANTS (HYMENOPTERA:FORMICIDAE) ASSOCIATED TO *Eurhizococcus brasiliensis* (HOMOPTERA:MARGARODIDAE) IN ROOT SYSTEM OF GRAPE VINE *Vitis* sp. IN SOUTHERN BRAZIL
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A survey of soil ants associated to ground pearl *Eurhizococcus brasiliensis* (Homoptera: Margarodidae) in the root system of grape vines *Vitis* sp. was carried out in southern Brazil. The method was to take systematic samples by the extraction of vines and collect insects from soil. Identification was made with the help of specialists using classical museological techniques. Results indicated following identifications: Dolichoderinae: *Dorymyrmex* sp.; *Linepithema humile* (Mayr); Formicinae: *Brachymyrmex* sp.; *Camponotus blandus* (Smith); *C. crassus* (Mayr); *C. melanoticus* (Emery); *C. renggeri* (Emery); *C. rufipes* (Fabricius); *Camponotus* sp.; *Paratrechina fulva* (Mayr); Myrmicinae: *Acromyrmex crassispinus* (Forel); *Crematogaster* sp.; *Pheldole aberrans* (Mayr); *Ph. megacephala* (Fabricius); *Ph. triconstricta* (Forel); *Solenopsis saevissima* (Smith); *Wasmannia auropunctata* (Roger); Ponerinae: *Hypoconerops* sp.; *Pristonopelta* sp.; Pseudomyrmecinae: *Pseudomyrmex* sp. Among these species, *Dorymyrmex* sp., *L. humile*, *Pheldole* spp., *S. saevissima* and *W. auropunctata* seem to have mutualistic relationship with *E. brasiliensis*. The species *L. humile* was, by far, the most frequent in the above mentioned samples.

17-068

BEHAVIORAL TIME BUDGET OF ADULTS OF *SMICRONYX GUINEANUS* VOSS (COLEOPTERA: CURCULIONIDAE) ON *STRIGA HERMONTICA* (DEL.) BENTH. (SCROPHULARIACEAE) IN SEMI-FIELD CONDITIONS

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The behavioral time budget of adults of *Smicronyx guineanus* Voss was studied in semi-field conditions. Pairs of weevils were observed on a 24-h cycle starting from 0800 to the next day at 0600 and repeated for 21 days in cages containing *Striga hermontica* (Del.) Benth plants parasitizing sorghum. Each female and male was observed every second hour. Occurrences for the various behaviors exhibited by the weevil were recorded. In decreasing order, the weevils were observed to exhibit standing still, feeding, mating, walking, moving and foraging. In addition, the difference in time spent on the different parts of the host plant was highly significant. These parts were classified in four groups. The weevil adults spent 46.8% of their time on the *Striga* inflorescence (bud, corolla and calyx). *Striga* plants were stratified with respect to distance from the base and categories were designated as upper, central and lower parts. *Smicronyx* adults spent 85.2% of their time in the upper stratum, 9.8% in the central stratum and 5% of their occurrences were in the lower third of the *Striga* shoot. They were more active in daytime, suggesting that the best period of day to sample these weevils in the field is from 0700 to 1100 or from 1600 to 1800.

Key-words: *Smicronyx*; *Striga*; behavior; time budget; activity; Burkina Faso.

Section 18

Urban and Stored Products Entomology

18-001

Introductory Lecture for Section 18:
CONTROL OF STORED FOOD PESTS IN ANTIQUITY

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The problem of preventing or controlling pest infestations in dried foodstuffs dates back to the Old Kingdom (~2575-2134 B.C.), when long-term storage was first practised in ancient Egypt. Early attempts of pest control, performed during the Middle (~2040-1785 B.C.) and New Kingdom (~1554-1080 B.C.) involved (a) divine charms and intimidations, (b) suitable design and location of food stores, (c) "antipathetic cures" and (d) pest-averting treatments. Most rational control measures (b,c,d) were prophylactic rather than curative, which resulted from the insistence of the priests to repel rather than exterminate harmful organisms. "Antipathetic cures" were based on the repulsive odour and insectistatic components of predatory animals, e.g. the feather lipids of entomophagous birds and the fur fat of rapacious cats. Protection of stored cereals was attained by addition of unthreshed grain (corn ears) and earth dust to the former, causing cuticular abrasions and water loss in detrimental insects. This ingenious procedure was probably invented by Jacob's son Josef (Koran 12,47) during the Hyksos period (1640-1532 B.C.). Fumigation by incense blends was frequently employed in sacred and profane premises. Disinfestation was actually paralleled by fulfilment of religious obligations. Several incense ingredients may act, according to concentration, as repellents, insectistatics or insecticides. The most effective ingredients were: myrrh (*Commiphora abyssinica*, *C. gileadensis*), frankincense (*Boswellia sacra*, *B. carteri*), essential oils of Citronella grass (*Andropogon nardus*, *A. winterianus*), Calamus roots (*Acorus calamus*), Cinnamon bark (*Cinnamomum ceylanicum*), dill seeds (*Anethum graveolens*) and Juniper berries (*Juniperus communis*, *J. phoenicea*). In ancient Rome, M.P. Cato (234-149 B.C.), L.J.M. Columella (1st cent. A.D.), M.T. Varro (116-27 B.C.) and Vitruvius Pollio (1st cent. B.C.) considered the proper location and design of food stores as a prerequisite for protecting foodstuffs against insect infestation. Granaries were raised above the ground in north-east direction (allowing air currents to dry and cool the cereals), advancing emigration of harmful species. Subterranean storage, causing suffocation to pest species, was an effective means of grain preservation. The above authors also recommended to apply an insectistatic mixture of "amurca" (aqueous extract of olives), clay and chaff to the interior of granaries. Moreover, Plinius Secundus (23-79 A.D.) and R.T.A. Palladius (4th cent. A.D.) suggested the addition of an insectistatic blend of "amurca", chalk and foliage of wormwood (*Artemisia absinthium*), Coriander (*Coriandrum sativum*) or fleabane (*Inula conyzia*) to stored grain, in order to minimize pest development. If compared with ancient times, the 20th century A.D. reveals a marked increase in the global amount of stored food, availability of a large variety and efficiency of storage protectants (biological, chemical and physical) as well as occurrence of pesticide-resistant species infesting stored food. The salient problems arising from food storage still resemble those encountered in antiquity, and improvement of these shortcomings remains a challenge for inventive minds.

18-002

PESTS, PATHOGENS AND PEOPLE: INTERACTIONS

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Pests, pathogens and people have at least one common denominator: food. Pest-food relationships can be categorized: accidental, opportunistic, obligatory. The obligatory pests tend to stay with the food; the opportunistic pests come and go as they please, sometimes visiting human food, sometimes visiting places that are grossly contaminated with bacteria, some of which may be pathogenic. The opportunistic pests carry the pathogens to human food; the obligatory pests spread them throughout the food. There is another much more common and more important way that people become exposed to contaminated food: the fecal-oral route. Typically, feces (bearing pathogens) find their way onto fingers, and fingers find their way into food. Fingers are the "transport hosts" of the fecal-oral route of enteric disease transmission. Pests are the functional counterparts of fingers; they, serving as transport hosts, pick up pathogens and carry them to food or food-contact surfaces. The load of pathogens may be small or large or any gradation in between. Even a large load may not infect a robust host. Even a small load may infect an immunocompromised host. No one should have to face the challenge of an infective dose, especially not in a hospital, the whole purpose of which is to help people get well, not to make them sicker. But put yourself in the role of a competent scientist who has studied all the evidence concerning pests as mechanical vectors of pathogens and has decided that there is nothing to it. But then you become seriously ill and must undergo a complicated surgery. You may choose one of two hospitals. One is known to have an effective program of integrated pest management; the other is known to have a thriving colony of cockroaches in every one of its food carts. Now, my dear rational, objective scientist, which hospital will you choose?

18-003

CAN FOOD PROVISION INCREASE THE EFFICACY OF BIOLOGICAL CONTROL AGENTS IN STORAGE SYSTEMS?

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Adult parasitic wasps generally require food both as an energy source for searching activities and for the production and maturation of eggs. The availability of sugar sources can increase the parasitoid's longevity, fecundity, and overall activity. The introduction of (artificial) sugar sources, consequently, provides a promising strategy to increase the effectiveness of natural enemies, during both crop cultivation and storage.

However, in those cases in which pest organisms benefit from the additional food source as well, introduction of nectar sources could backfire. Therefore, the effects of nectar provision on both the pest insects and their antagonists should be addressed.

In the present study, the effect of artificial food sources on the longevity and fecundity of the cowpea weevil *Callosobruchus chinensis* and its parasitoid *Anisopteromalus calandrae* was investigated. Weevils and parasitoids were either provided with water only, or exposed to water in combination with either honey, unfested beans, *C. chinensis* infested beans (host feeding), or infested beans plus honey.

Nectar feeding was found to increase both longevity and lifetime fecundity of *C. chinensis*, while the weevil's lifespan was not affected by the other treatments. Both honey and weevil infested beans increased the parasitoid's longevity dramatically. Parasitoid lifetime fecundity was found to be primarily affected by host feeding.

The implications of these findings for biological control of storage pests will be discussed along with possible strategies for making food application selective.

18-004

POPULATION COMPOSITION AND IMMIGRATION -
IMPLICATIONS FOR SELECTION OF CONTROL METHODS FOR
PHYCITINE MOTHS IN A FOOD PROCESSING PLANT.

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Knowledge of the nature, extent and movement of populations is important in the selection and likely success of control measures. A study was made of the seasonal occurrence and species composition of phycitine moth populations in and around a breakfast cereal manufacturing plant in the State of Victoria, Australia. Moths inside the processing facilities and outside in the surrounding district were monitored using pheromone baited sticky flight traps. Five species were captured; *Plodia interpunctella* and four *Ephesia* spp.: *E. cautella*, *E. kuehniella*, *E. elutella* and *E. figulilella*.

In the processing facilities, the main pest species was *E. cautella*. A few *E. kuehniella* and *P. interpunctella* were also caught. Catches varied seasonally but remained relatively constant all year.

Catches in outdoor traps, placed in surrounding towns and country, peaked in summer and stopped briefly mid-winter. *P. interpunctella* was the dominant species in urban residential areas. *E. kuehniella* and *E. elutella* were particularly associated with feed mills in the area. Very few *E. cautella* were caught outdoors. In contrast, *E. figulilella* was only caught at outdoor locations.

E. cautella in the manufacturing plant appeared to be an isolated population. Moths captured outside were almost always other species. While active for much of the year, these species either did not enter or did not thrive in processing facilities. Novel control measures against moths in food processing plants, such as using parasites and mating disruption, which would be compromised by significant pest immigration, could prove efficacious in this case.

18-006

LACHESILLA QUERCUS (KOLBE) (PSOCOPTERA:
LACHESILLIDAE) A NEW PEST OF GRAIN STORES IN AUSTRALIA

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Since November 1992, the psocopteran *Lachesilla quercus*, an insect previously unknown in Australia, has been found infesting coastal grain storage facilities in the south west of Western Australia. More recently it has also been found in grain stores on the Eyre peninsula of South Australia.

In these areas *L. quercus* has become a pest of a status apparently unrecorded in other countries in which it is known to occur. It has the ability to thrive in numbers in places kept sufficiently clean to prevent the establishment of most other storage pests. *L. quercus* appears to browse on minute particles of dust that settle on surfaces as a result of grain movement. Undisturbed populations produce large quantities of silken webbing under which they live. This quickly becomes unsightly as it gets covered with dust. Large numbers of dead insects can accumulate, together with deposits of fine black frass, on ledges below infestations. Workers are distracted and sometimes distressed when working in areas in which large numbers are flying.

Changes in pest control practices in the Australian grain industry in response to market demands may have inadvertently favoured this insect. Market demands for low or no insecticide residues has meant the reduction or cessation of spraying of store structures or the admixture of insecticides into grain. Grain dusts would now likely be free of insecticide residues and available for exploitation by this insect.

It is not clear how long this insect has been in Australia or if it is yet established in natural habitats on the continent. However, its current status in grain storages is a very recent phenomenon.

18-005

TRAIL FOLLOWING BEHAVIOR IN THE GERMAN
COCKROACH, *BLATTELLA GERMANICA* (L.)
(ORTHOPTERA: BLATTELLIDAE)D. M. Miller, P. G. Koehler¹, R. S. Patterson²Department of Entomology and Nematology, University of Florida,
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The ability of German cockroaches (adult males, adult females and late instar nymphs) to use an extract of their fecal material as a trail pheromone was evaluated in visually deprived arena tests. Using the Dynamic Animal Movement Analyzer program we were able to determine that $\geq 90\%$ of the cockroaches correctly oriented toward and moved in the direction of a preapplied trail of fecal extract upon release into the test arena.

In tests where cockroaches were provided with two identical plexiglass edges to follow either along a trail of fecal extract or along a trail of methanol (control), 90% of the adult male cockroaches correctly selected and followed the trail of fecal extract accurately. Ninety percent of adult females also followed the fecal extract trail accurately as well as 93% of the late instar nymphs.

When no edge was present and all cockroaches had a 360° choice of directional orientation upon leaving the harborage, trail following accuracy was reduced for all three cockroach groups. However, 80% of the adult males still followed the trail of fecal extract accurately as well as 60% of the females and 47% of the nymphs.

18-007

Morphology and Distribution of the Sensilla of Antennae,
Maxillary and Labial Palps, and Tarsi of *Callosobruchus*
maculatus and *C. subinnotatus*George N. Mbata, Srinivas Chinta, and Sonny Ramaswamy
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Abstract: Sensilla on antennae, maxillary and labial palps, and tarsi of *Callosobruchus maculatus* and *C. subinnotatus* were studied using light and scanning electron microscopy. Six types of antennal sensilla occur in both species. Type 1 are 86-92 μm long and 2.6-2.9 μm in diameter, and have longitudinal ridges and a conspicuous basal socket. These sensilla occur in rings at the distal margin of each antennule; the distal antennule bears two such rings. Type 2 sensilla are found on all segments and are 38-41 μm long, with a well developed basal socket, and less conspicuous cuticular ridges. Type 3 sensilla are numerous and found only on flagellar segments. These range in length from 36-41 μm , have no socketed base, and are devoid of cuticular ridges. Type 4 are basiconic sensilla, 23-28 μm in length, smooth surfaced, lack a membranous base, numerous and present only on flagellar segments. Types 5 and 6 sensilla are short (<10 μm), few, and are found along with type 4 sensilla in groups distally on the distal antennule. Type 5 sensilla are basiconic, have a fluted surface and a pitted basal socket. Type 6 are basiconic pegs, and are devoid of basal socket and cuticular sculpturing. Types 1 and 2 are sensilla chaetica, type 3 are trichodea, and types 4, 5 and 6 are sensilla basiconica. Maxillary and labial palps in both species have three types of sensilla: type 1 (32-35 μm long), type 2 (21-26 μm), and type 3 (2.9-3.8 μm). Types 1 and 2 lack surface sculpturing, have basal sockets, and are found on maxillary and labial segments. There are about 20-25 type 3 sensilla, and in females, maxillary tip sensilla are fluted. Tarsi of both species have 4 types of sensilla; type 1 sensilla (43-48 μm long), type 2 (26-44 μm), and type 3 (18-26 μm); type 4 (26-36 μm) are curved and present only on the pad like structures on the second tarsomere. Types 2 and 3 have socketed, flexible bases.

18-008

CARBOHYDRATE FOOD-SOURCES OF COPROPHAGOUS SEPSIDAE (DIPTERA) IN AN URBAN SEWAGE-PLANT

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Themira putris (L., 1758) (Diptera: Sepsidae) occurs in a sewage plant during the whole vegetation period with several generations. These coprophagous flies utilize varied sources for vital carbohydrate food:

- 1) The nectar from blossoms of a wide range of plants belonging to different families (e.g. Brassicaceae, Compositae, Geraniaceae).
- 2) During the midsummer mass occurrence the honeydew of different species of aphids (Homoptera: Aphidina) (e.g. *Aphis fabae* Scop., *Brachycaudus cardui* (L.), *Uroleucon sonchi* (GEOF.), *Rhopalosiphum padi* (L.)). *T.putris* sucks up the deposited honeydew. *T.putris* also absorbs accessible honeydew deposits from aphids (e.g. *Eucallipterus tiliae* L.) not native to the habitat of *T.putris*.
- 3) In late summer *T.putris* sucks up the honeydew of scales (Homoptera: Coccoidea) from infested plantparts.
- 4) At the end of the summer and beginning of autumn *T.putris* absorbs the sugar containing secretion produced by ripping ergots (Fungi: Ascomycetes) on ears of wild grass.

18-010

MAIZE STACKBURN IN SUB-SAHARAN AFRICA: CAUSES AND CONSEQUENCES

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During the last decade in sub-Saharan Africa, the discoloration of stored maize as a direct result of heat build-up in the interior of bag stacks, has emerged as a significant threat of food security. As a result of such phenomena, internationally called "stackburn", the maize is then regarded by users as less suitable for food milling and is nutritionally altered, reducing its value as human or animal food. The possible causes for stackburn are under investigation by ecological studies on interactions between grain conditions, storage microclimate and insect and fungal development in Zimbabwe, Ghana, United Kingdom and Portugal. The introduction of woven polypropylene sacks and its associatior with increased levels of insect infestation remains the most likely cause.

18-009

TAPINOMA MELANOCEPHALUM FABR. (FORMICIDAE, DOLICHODERINAE) IN BUILDINGS OF GERMANY - OBSERVATIONS TO ITS BIOLOGY AND CONTROL

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This tropical ant was found 1982 in Halle-Neustadt (kindergarden), 1985 in Rostock (bakery) and was also observed and controlled in Berlin 1994-1996 (dwelling-houses). Important exterior characteristics are: size 1,3-1,5 mm (♀♀), 2,3-2,5 mm (♀♀,♂♂), petiolus is not scale-like, gaster with 4 from dorsal visible segments. Colour: caput blackbrown to black; gaster is cream to brownyellow (♀♀), brownish (♀♀), blackbrown to black (♂♂), coloured. Preferred food: lieing about food, dead flies and cockchroaches, cake, milk, sweets. This species was fed in labratory with mixture from yolkpowder, biskuitmeal and honey, dead insects. The inquiry of attack-locality results with pig-lever and saturated sugar-solution. Baits-boxes were placed on the attack-locality directly. It was distinctly, that Maxforce-Bait (Hydramethylnon) was visited middling. Baits with a mixture of peanut-fat and boric or of yolkpowder, honey and boric were attractive about a longer time. The infestation was destroyed with these baits, but it continues in only one flat. A big *T.melanocephalum*-colony was found in this flat under very moist and mouldy bathroom-floorsettling from this species. *T.melanocephalum* was introduced likely with tropical flowers in dwelling houses, then a flower-friend lives there.

18-011

INFLUENCE OF THE CO₂ AND TEMPERATURE INCREASE BY SITOPHILUS ZEAMAI MOTCH. (COLEOPTERA: CURCULIONIDAE) POPULATIONS IN CAUSING MAIZE STACKBURN

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The respiration rate of insects infesting stored maize while producing heat in the maize stock grains, may have the potential to contribute for the stackburn. In the present work the authors are concerned with the rate of carbon dioxide production of *Sitophilus zeamais* at various levels of infestation at 28° C, as a way to assess respiration rates and its effect on the rise of temperature.

18-012

REPRODUCTIVE BIOLOGY IN RELATION TO TRANSPORT OF DICHOTOMOUS SPERMATOZOA IN POTATO TUBER MOTH *PHTHORIMAEA OPERCULELLA* ZELLER (LEPIDOPTERA: GELECHIIDAE)

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Potato tuber moth is a serious pest of potato in the stores as well as in the fields in India. Sometimes it has also been reported to attack other solanaceous crops. This moth shows multiple mating behaviour. More than 70% of the moths mate twice in their life span. Average time of mating vary with temperature with a mean of 2.27 ± 0.25 hours in summer and 4.22 ± 0.19 in winter months. Two types of sperms are produced by this moth in the testes in bundle form and both are transported to the female tract during mating. The migration of apyrene spermatozoa from testes to vas deference starts earlier than eupyrene ones that also in pupal stage. In the vas deference the eupyrene sperms retain their bundle form whereas apyrene sperm bundle gets dissociated into individual sperms. Both types of spermatozoa are packed into a spermatophore in the ejaculatory duct and is deposited in bursa copulatrix of female within 25 to 60 minutes of mating period (at $27 \pm 2^\circ\text{C}$) in the next 30 to 50 minutes eupyrene and apyrene spermatozoa get lodged separately into utriculus and lagena lobes of the spermathecae. Later they get mixed up in these lobes. The vigorous movements of the apyrene spermatozoa in the bursa copulatrix and other parts seem to be the force for separating eupyrene bundle into the individual ones and transporting them.

18-014

THE BIOLOGY OF THE PARASITOID *LAELIUS PEDATUS* (SAY) (HYMENOPTERA: BETHYLIDAE), AND ITS POTENTIAL FOR THE BIOLOGICAL CONTROL OF *TROGODERMA GRANARIUM* EVERTS AND *TROGODERMA ANGUSTUM* (SOLIER) (COLEOPTERA: DERMESTIDAE)

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Laboratory studies were conducted to assess the potential of the ectoparasitic wasp *Laelius pedatus* (SAY) for controlling *Trogoderma granarium* EVERTS and *Trogoderma angustum* (SOLIER). The female *L. pedatus* paralyzes the host larvae and deposit its eggs. The stung larvae are completely or partially paralysed. The total number of eggs laid, and the larvae paralysed, were significantly lower on *T. granarium*. One female of *L. pedatus* was able to paralyse on an average 50 larvae of *T. granarium* and 150 larvae of *T. angustum*. All paralysed larvae of *T. angustum* died after 14 days, but 40 % of the paralysed larvae of *T. granarium* recovered after 4 to 5 weeks and continued their development to adults. The number of host larvae killed by paralysis was higher than the number killed by parasitisation. If no males are available the female wasp deposits eggs by arrhenotoky reproduction. These eggs develop to males only. A mated female was able to deposit fertilized eggs for a maximum period of 14 days after copulation with a hatching rate of 75 - 83 %. The sex ratio of the progeny of mated females was 1:2 (male:female). An average of 52 eggs were deposited on *T. angustum* by a single female of *L. pedatus*. The duration of the development was influenced by temperature and lasted 31 - 37 days at 28°C . The lifetime of the wasps depended on the food quality and required 1 to 2 and 4 to 5 weeks, respectively for males and females supplied with host larvae only. When the wasps were supplemented with honey, adult life of males and females was extended to 4 and 7 weeks, respectively. Unfed females and males lived for less than 14 days.

At the parasitoid: host ratio of 1: 25, *L. pedatus* reduced the population of *T. granarium* by 73.5 % within 6 weeks.

18-013

POSSIBILITIES OF CONTROLLING PESES FOLLOWING THE PHASE-OUT OF METHYL BROMIDE

S. Navarro

ABSTRACT NOT RECEIVED

18-015

THE OUTLOOK FOR USING OF JUVENILE HORMONE ANALOGUES (JHA) AND CHITINE SYNTHESIS INHIBITORS (CSI) FOR THE DEPRESSION OF URBAN MOSQUITO POPULATIONS SIZE

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The existence of favourable conditions for mosquitos development and reproduction in large towns (flooded basements of dwelling houses, barrels, sewage, capacities for water collection, ponds) have result in the sharp increasing of mosquitos population size, *C.p. molestus* Forsk especially, and in the "problem of urban mosquitos" arising.

It is proved a possibility of depression of urban mosquitos population size in 6 large towns with the help of JHA and CSI. CSI Dimilin 25% w.p. and Alcistin 48% e.c. and 25% w.p. after employment of 40-60 g of AI/hectare have prevented winged mosquitos exclusion during 23 - 59 days at the expense of larvae development disturbances during moulting passage. JHA Altozid SR-10 (50 g/hect) ensured mosquitos exclusion absence during 32-35 days at the expense of anomalies under imago moulting (pupa-imaginal forms, defective imago). Metopren 4.7% (1 briquette/10 m²), Metopren 6% (6.4 g of granules/m²) and Sumilar (10 g of 0.5% granules/m²) ensured mosquitos exclusion absence during 46-50, 40-50 and 32-34 days. The employment of granules and tablets is the most perspective since its effect is attained at the expense of slow preparation diffusion into the water; the latter reduces xenobiotics content in the environment and leads to the lowering of desinfection costs.

18-016

RELEASE OF *TRICHOGRAMMA EVANESCENS* AS A COMPONENT OF AN INTEGRATED PEST MANAGEMENT PROGRAMME IN ORGANIC FOOD BAKERIES AND STORES (HYMENOPTERA: TRICHOGRAMMATIDAE)

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The pyralids, *Ephestia kuehniella* ZELLER and *Plodia interpunctella* (HÜBNER), are the major stored product pest moths in the food processing industry in Central Europe.

Field trials with *T. evanescens* egg parasitoids were conducted in organic food processing industry and the linked retail-trade. During the field trials, the parasitoids were not affected by chemical control since in these branches synthetic chemical insecticides are not applied.

The pest populations were monitored in 1994 and 1995 using TDA baited funnel traps and delta traps. Integrated pest management including biological control with *T. evanescens* and a hygiene programme started in 1995. The egg parasitoids were released weekly from cardboard egg-cards in the areas of the bakeries and stores where moth infestation was expected.

Compared to the year before without integrated control under similar weather conditions, the number of moths trapped in 1995 was reduced by one third.

The control programme did not affect the production process. The acceptance of biological control by the workers and retailers was very encouraging. The programme will be continued and extended in 1996.

18-018

ACOUSTICAL/ELECTRONIC DETECTION AND AUTOMATED MONITORING OF STORED-PRODUCT INSECTS

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Acoustical and electronic technology has been applied to aid in the management of stored-product insect pests. A computer-based system was developed to quantify infestation of internally feeding larvae by ascertaining the number of sound source locations in a grain sample. This is accomplished using a cross-correlation and cluster analysis algorithm applied to data acquired from an array of acoustical sensors mounted in a sample container. This system was quite accurate for counting low-level infestations of late-instar *Sitophilus oryzae* larvae, except when the insects were very near one another. The device is also very accurate for determining uninfested samples. An automated system was developed to provide continuous monitoring of insects at multiple sites within large volumes of stored-products and to remotely display acquired data indicative of infestation levels at these sites. The system uses infrared beam sensors to count insects as they drop through pitfall grain probes distributed throughout the storage volume. The collected data are transmitted to a central computer via a tree structured data transmission network capable of efficiently connecting to thousands of probes in a large grain bulk. This system was very accurate for counting many commonly occurring species of adult stored-product insects in laboratory trials using both wheat and maize substrates.

18-017

BIOLOGICAL CONTROL OF TERMITES - *METARHIZIUM* AS A MYCOINSECTICIDE

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Recent research has shown that the insect pathogenic Hyphomycete fungus *Metarhizium anisopliae* is a promising biological insecticide for the major pest species of subterranean termites in Australia. From an Australia-wide survey of termite nest mounds and feeding material of 42 species of termites, more than 100 different isolates of *Metarhizium* were obtained. The most virulent fungal strains were selected by a bioassay based on the natural grooming behaviour of termites. Temperature and humidity tolerances of the isolates *in vitro* were tested, and the best isolates were assessed in laboratory termite "mini-colonies". Field trials using the most effective isolate, FI610, by blowing conidia directly into mounds of *Coptotermes acinaciformis*, *C. lacteus* and *Nasutitermes exitiosus*, have shown that up to 100% control of the colonies is achievable with as little as 1g of pure conidia, depending on correct application of the conidial formulation and the time of the year in which it is applied. The fungal conidia persist within the termite nest environment for at least 2 years. Indirect treatment of colonies by treating termite feeding sites, including house timbers, with *Metarhizium* is currently being assessed. Australian termites can detect *Metarhizium* and therefore further studies on behaviour and implications for control strategies using *Metarhizium* are being evaluated. Suspensions of *Metarhizium* conidia sprayed directly onto and/or into timber structures could be used to protect against termite damage. *Metarhizium* is also being tested as a barrier treatment using conidia mixed into soil; over 2 years protection of timber has been achieved in SE Australia, whereas a shorter protection time resulted at tropical field sites.

18-019

MORTALITY OF TWO STORED-PRODUCT INSECT SPECIES EXPOSED TO MICROWAVE RADIATION

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Microwave radiation emitting at 2,450 MHz frequency was used continuously and intermittently to evaluate the mortality of stored-product insects *Tribolium confusum* and *Plodia interpunctella* as a function of exposure time and incident power. The survival kinetics was highly quadratic ($0.82 \leq R^2 \leq 0.99$) for each of the growth stages studied. The target theory was invoked to model the dose-survival relationship. Mortality was dependent on insect development stage at any microwave power input. Insects were killed by overheating and the lethal medium temperature for both species was estimated at about 80°C for the most resilient growth stage. Intermittent exposures were generally more effective in killing insects of both species compared with those of continuous irradiation. The fraction survived increased as the moisture content of the medium increased from 6 or 9 to 12%. The insects in the mobile state were observed to move towards the surface from inside the nutrient medium during irradiation. The authors could not detect any generation of "hot spots" in the medium despite concern of scientists involved in industrial heating of materials by microwaves.

18-020

EFFECTS OF GAMMA RADIATION ON THE DEVELOPMENT OF CIGARETTE BEETLE (*LASIODERMA SERRICORNE* (F.))

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The effects of gamma radiation doses on the metamorphic stages of Cigarette beetle *Lasioderma serricorne* (F.). were studied in the laboratory of Division of Entomology, Indian Agricultural Research Institute, New Delhi. Four different age groups viz., 1,2,3,4 days old eggs were irradiated between 0.5 to 4.0 krad doses. Results showed that older eggs were more radio resistant and hatching reduced with the increase in dose. A dose of 4.0 krad completely prevented the hatching of eggs. The development of adults from treated eggs was prevented by 3.0 krad dose. When 20 days old larvae of this insect were irradiated between 1 to 20 krad doses of gamma radiation, 8.0 krad dose completely prevented the pupal formation, while the doses between 3-6 krad prolonged the larval period, delayed pupation and adult emergence. The adults emerged from irradiated pupae survived in lesser numbers and a dose of 60 krad completely prevented the adult emergence. Freshly emerged adults when irradiated between 10-100 krad doses the survival time of irradiated adults decreased with increasing dose. A dose of 70 krad was sufficient to achieve complete mortality of adults 15 days after treatment. It is concluded that egg was the most radiosensitive stage.

18-022

COMBINED EFFECTS OF SILICA AEROGELS AND INSECT GROWTH REGULATORS AGAINST *SITOPHILUS ZEAMAE* MOTCH. (COLEOPTERA: CURCULIONIDAE) INFESTATIONS

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Laboratory experiments were conducted to determine the effectiveness of two silica aerogels (Neosyl TS and Gasil 23D) and two Insect Growth Regulators (IGRs), (diflubenzuron and fenoxycarb) when used alone and in combinations against populations of *S. zeamais*.

The silica dusts applied to grain were both effective but there were differences between the two materials.

The IGRs treatments showed satisfactory effect in preventing the FI emergencies of *S. zeamais*. However, in order to minimise the chemical residues left on the food grains a reduction in dosages of the IGRs components and its effects with the joint action of the silica dusts is under research.

18-021

INSECT DISINFESTATION OF NUTS AND DATE BY IRRADIATION IN ISLAMIC REPUBLIC OF IRAN

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Abstract

Iran produces annually about one million tonnes of nuts and Date. The average storage losses caused by insects are found to vary from 30-35% despite the traditional methods of pest control. The insects identified as causing considerable damage to the above - mentioned agricultural products include. *Plodia interpunctella* (Hbn) and *Oryzaephilus surinamensis* (L.) The dose range for the control of different developmental stages.e.g.egg larval, pupa and adult of the above mentioned insects were found to vary from 0.3 to 0.45 KGY and 0.35 to 0.5 KGY, respectively.

18-023

INSECTICIDAL ACTIVITY AND MODE OF ACTION OF A NEW SYNTHETIC PYRETHROID, IMIPROTHRIN

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A new synthetic pyrethroid, imiprothrin; [2,5-dioxo-3-(2-propynyl)-1-imidazolidinyl] methyl (1*R*)-*cis,trans*-chrysanthemate shows the highest knockdown activity against household insect pests, especially against cockroaches among the known pyrethroid compounds.

The toxicological and electrophysiological studies for imiprothrin were carried out using cockroaches. We will discuss the difference in the mode of action between imiprothrin and the other pyrethroids.

The new method for the evaluation of knockdown activity will be also discussed.

18-024

INVESTIGATIONS ON THE SUITABILITY OF SILAFLUOFEN TO CONTROL NOXIOUS ANTS, WASPS AND TERMITES

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Within the Order *Isoptera* and *Hymenoptera* there are some social insect pests, for example termites such as *Coptotermes formosanus* or *Mastotermes darwiniensis*, leaf cutting ants such as *Atta*- and *Acromyrmex*- species, and wasps such as *Vespa vulgaris* and *Vespa analis*, which destroy wooden buildings, damage important crops, beekeeping and tropical forestry or jeopardize and attack human beings.

With silafluofen (Neophan®, Silonen®), which has a „delayed action kill“ and, if applied specifically, it is possible to combat pest insects of these kinds „systemically“.

Quick kill of individual foragers of social insects, such as ants, wasps and termites does not affect the main colony. However, if silafluofen is mixed with an insect attracting ingredient, the foragers carry the toxicant-attractant formulation back to the home colony where it is shared by larvae, workers and queen.

With silafluofen (0.0005% - 0.0025% a. i.) sprayed upon taro, sweet potato, manioc and coffee, whole hives of leaf-cutting ants are destroyed.

Wasp numbers can be reduced by destroying nests using silafluofen 0.005% a. i. in a honey : water bait (1:5).

Hives of the Australian subterranean termite can be destroyed „systemically“ when fed with a wooden block which was dipped into a silafluofen solution (0.004% a. i.).

The laboratory experiments with ¹⁴C-labelled silafluofen have shown, that silafluofen is a versatile insecticide to control noxious ants, wasps and termites.

18-026

TARGETED DELIVERY OF MOSQUITO LARVICIDES FROM MATRICAP™ COMPOSITIONS

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A novel formulation system for controlled delivery of pesticides was developed for targeting a variety of aquatic organisms that occupy specific surface, subsurface or both surface and subsurface areas of a water column. Polymer/nonpolymer-base formulations consisted of admixtures of one or more insecticides, and one or more matrix and encapsulation/coating components, with or without binder components.

A series of short and long-term bioassays demonstrated positional distribution of bioactive agents such as *Bacillus thuringiensis* var. *israelensis* (B.t.i.) or methoprene, or a combination of B.t.i. and methoprene from powdered, granular or agglomerated compositions in zones of a water column that were optimal for controlling immature stages of *Anopheles*, *Aedes*, and *Culex* species of mosquitoes that typically feed and/or orient in different horizontal and vertical areas of an aquatic habitat. In general, bioassays against mosquito larvae indicated that the encapsulation level, controlled-release rate, and controlled-release profile of an insect growth regulator or bacteria were dependent on the type and concentration of admixing components utilized in the powdered, granular or agglomerated compositions. The duration of delivery of mosquito larvicides was shown to be mainly related to the type and concentration of coating(s) utilized in a controlled-delivery composition.

Controlled-delivery Matricap™ technology was also applicable to terrestrial pest control. As an example, novel polymer-pesticide (e.g., organophosphate or growth regulator) bait stations were developed for prolonged control of adult and immature stages of the German cockroach *Blattella germanica*.

18-025

THE EFFECTIVENESS OF DIATOMACEOUS EARTH AGAINST STORED-GRAIN INSECTS PESTS IN FARM STORAGES.

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Diatomaceous earth (DE) has gained interest as a grain protectant as it is non-toxic to mammals, and it does not affect end use quality (baking, malting, or pasta production). However, DE use has been limited because it reduces grain bulk density and must be used at high concentrations, 500 to 3500 ppm (parts per million), to be effective. We have developed a new diatomaceous earth-based insecticide, Protect-It, that is approximately 2-3 fold more effective than currently available diatomaceous earth grain protectants. We present the results of field trials conducted in southern Manitoba.

Field trials were conducted at one site in 1994 using 3 treatments (0, 50 and 300 ppm dust) and at three sites in 1995 using 4 treatments (0, 75, 100 ppm dust, 100 ppm aqueous spray). We used 27 - 80 tonne capacity metal storage granaries with 16 - 40 tonnes of wheat in each granary. To insure infestation, rusty grain beetles (*Cryptolestes ferrugineus* (Stephens)) and red flour beetles (*Tribolium castaneum* Herbst) were released on the top of the wheat bulks. Insect populations were measured using probe pitfall traps and by extracting insects from wheat taken from the granaries.

Cryptolestes ferrugineus populations were consistently controlled, over 90% reduction compared to control granary populations, by treatments at 75 ppm or greater. *Tribolium castaneum* populations were reduced by 100 ppm dust application, and controlled at 300 ppm. Neither species were consistently reduced at 50 ppm, and *T. castaneum* was not reduced at 75 ppm or 100 ppm aqueous spray applications.

Grain bulk density (kg/hL) was reduced by 2.1 at 50 ppm, 2.2 at 75 ppm, 2.3 at 100 ppm, 2.1 at 100 ppm spray application and 4.6 at 300 ppm. The grain grade in treated wheat was not reduced due to bulk density reduction, but grain with bulk densities at the limit of a grain grade could have reduced grades due to bulk density reduction.

18-027

QUANTITATIVE LOSS ANALYSIS OF THE DAMAGE CAUSED BY *LEPISMA SACCHARINA* LINNÉ (ZYGENTOMA: LEPISMATIDAE) ON DIFFERENT BOOK-MATERIALS AND THE EFFICACY OF BORIC ACID AND DIATOMACEOUS EARTH TO CONTROL THIS HOUSEHOLD PEST

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Silverfish are common in bathrooms all over the world. In most cases nobody takes care about these harmless guests. But in a library or in a museum this pest can cause considerable damage to valuable historical books, letters, documents, paintings and pictures.

In laboratory experiments the losses caused by *Lepisma saccharina* to different book-materials such as hand-made paper, tissue paper, vellum, leather, linen etc. were quantified. The efficacy of two non toxic powders, boric acid and diatomaceous earth to control *L. saccharina* was also tested. Boric acid gave good results in controlling *L. saccharina* under all the conditions tested. Diatomaceous earth was less effective especially under humid conditions. Quantitatively, 100 silverfish destroyed about 1 g a year of some of the book-materials tested, equal to 100 cm² to 700 cm² depending on the type of material.

18-028

EMPLOYMENT OF HEAT AND COLD AGAINST PEST INSECTS IN STORAGE

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The susceptibility of insects towards exposure to heat and cold is generally known since long time. Compared with chemical control the application of heat and cold to disinfest storages, museums and collections from pest insects was much more expensive and rarely chosen in practice. Due to changing public awareness and attitude including more intense measures to ensure the safety of employees, the treated goods and the environment, chemical treatments are increasingly more expensive. This new consideration opens the horizon for the implementation of the above mentioned control methods into an integrated system of pest control with biological, physical, chemical and technological procedures in storage.

The paper presents data from literature, laboratory and practical results of the use of heat and cold to control pest insects.

Temperatures in the range of 50°C are sufficient in flour mills for control of insects within a few hours. The possible destruction of electronics and other sensible materials sets a narrow margin for efficacy against pests and avoidance of damage as far as the temperature regime is concerned. New systems with use of accurately regulated relative humidity try to include heat control as a method against material destroying insects in precious artefacts. Heat treatment is discussed as an alternative for use of methyl bromide, which is considered to be replaced in pest control wherever possible.

With cold it is more the aspect of high costs which limits the application in such cases where time is very limited and the goods to treat are very sensible and of high value. Practical results with liquid nitrogen supporting the applicability cold are presented.

18-030

BIOLOGICAL EFFECTS OF SOME NATURAL AND CHEMICAL COMPOUNDS ON THE POTATO TUBER MOTH, *PHTHORIMAEA OPERCULELLA* ZELL. (LEPIDOPTERA: GELECHIIDAE).Aziza Sharaby¹, M.M. El-Missiry², S. Abdel-Hamed³
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The olfactory reaction of larvae and moths was investigated towards 18 oils (6 natural oils and 12 commercial chemical oils). Some of these oils as peppermint and camphor (natural oils), eugenol and camphene (commercial oils) were repellent to both larvae and moths. Other oils as straw berry and D-Limonene were attractive to both larvae and moths.

Eugenol and peppermint oils, each at the 0.01 % conc. caused significant depression in the fecundity of moth and decreased the percentage of egg hatchability at 1 %. Eugenol oil, however, was much more effective than peppermint oil.

Dried (leaves or fruits or seeds) powders of 14 different plant species were tested in different concentrations with talcum powder (carrier material) against egg deposition. Results indicated that:

Dried powders of *Allium cepa*, *Curcuma longa*, *Colocasia antiquorum*, *Ocimum basilicum*, *Dodonaea viscosa* and *Thuja orientalis* displayed a high significant role in reducing egg deposition. The most impressive effect was displayed by powders of *Dodonaea viscosa* and *Allium cepa*, which caused the highest depression in egg deposition as well as in the emerging offspring.

Ethanollic extracts of *Pituranthos tortosus* and *Iphiaona*, scabra caused the maximum inhibition of egg hatchability, followed by *Curcuma longa*.

18-029

RED IMPORTED FIRE ANT (HYMENOPTERA: FORMICIDAE) CONTROL: SHOULD BAITS OR STRATEGIES BE IMPROVED?

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We used field tests and population modeling to compare efficacy of commercial baits used in red imported fire ant (*Solenopsis invicta* Buren) control. Check and treatment plots (each >0.4 ha) were established at 54 sites in southern Arkansas using twice-yearly (June and October) broadcast applications of commercial hydramethylnon and fenoxycarb baits applied at a rate of 0.68 kg/ha. Ant colonies were counted monthly within 0.1 ha subplots. For analysis, colonies were placed into five size classes and colony density (per 0.1 ha) and total percentage coverage of colony foraging territories in plots were estimated. Computed values for colony density and territory coverage were averaged over the period July 1992 to June 1993, producing a new variable called active period average (APA). These APA values for check and bait plots were averaged spatially as well, and used to assess the efficacy of this bait strategy. For check plots, mean mound density (and its standard error) was 26.0 (± 3.4) and mean territory coverage was 21.5% ($\pm 3.3\%$). For hydramethylnon these measurements were 5.1 (± 1.1) and 2.6% ($\pm 0.6\%$), respectively, and for fenoxycarb they were 5.1 (± 1.1) and 2.1% ($\pm 0.7\%$), respectively. Population modeling yielded 2.6 mounds per 0.1 ha as the lower limit of density under twice yearly 100% suppression. Observed mean efficacy of baits in reducing colony density and territory coverage was 80% and 84%, respectively; there were no statistical differences between baits. Therefore, applied twice per year, these baits were effective at killing ants. The model of fire ant population growth, with 100% population suppression twice a year, produced a theoretical maximum reduction in colony density for the year of 90% due to rapid recolonization of treated areas. Because the 80% reductions achieved in field experiments are close to the calculated theoretical upper limit of 90%, the strategy of using twice-yearly applications of baits for managing fire ant populations appears to be reaching its maximum potential. Consequently, alternative strategies for controlling fire ants seem to be imperative.

18-031

CONTROL OF INSECT PESTS IN STORED GRAIN WITH CONTROLLED ATMOSPHERES RICH IN CARBON DIOXIDE AT TEMPERATURES BETWEEN 0°C AND 40°C

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Controlled Atmospheres (CA's) low in oxygen and/or high in carbon dioxide have been registered for stored product protection purposes in many countries during the last decade. Due to the high density of carbon dioxide it is sufficient to secure the desired CO₂-content at the top of a treated grain storage. This allows treatments not only in silo bins but also in flat storages sealed with gasproof plastic liners. The insecticidal effect of carbon dioxide fumigations does not depend solely on the replacement of oxygen. This allows for higher residual oxygen contents than in nitrogen treatments.

Grain temperatures have a major impact on the time needed for a successful disinfestation. The grain temperatures in an unclimatised grain store may vary considerably over the year and may range from around 0°C in late winter up to 35°C or even higher in late summer.

To study the influence of product temperature on the efficacy of atmospheres containing 60 % or 90 % of carbon dioxide in air, all developmental stages of the granary weevil *Sitophilus granarius* (L.) were exposed to these two gas mixtures at temperatures between 0°C and 40°C. Longest lethal exposure times of 49 days were measured at 5°C while probably the cold is responsible for a reduction to 35 days at 0°C. At 35°C lethal exposure times were reduced to 7 days and at 40°C to less than 2 days. Over the whole temperature range no significant deviations could be found in the efficacy of 60 % and 90 % CO₂. The results are compared to data of experiments using different species.

18-032

Biogeneration of modified atmosphere in small grain bins for the control of storage insects.

M. Calderon, N. Paster and Miguel Mora

Abstract

The principle of his method is to use a deterioration of waste plant material in small containers (biogenerators) for the production of high CO₂ concentrations and the consumption of O₂ (resulting on low O₂ contents). The produced, in the biogenerator, lethal to the insects modified atmosphere is transferred in the grain bin in which insect infestation takes place. The three parameters of the system were investigated and the following findings were noted: The Biogenerator may be of any volume depending on the quantity and the type of the waste plant material (the substratum) and the size of the grain bin. The substratum. It was concluded that wet substrata (orange peels, bananas, etc) under anaerobic conditions, produced markedly more CO₂ than dry materials (like wheat bran). The CO₂ production was notably large in the case of sugar cane. The grain bins were of capacity of 200 - 1000 kg of grain. It should be noted that this method was successfully demonstrated in Costa Rica (Central America).

18-034

RESISTANCE TO PHOSPHINE IN STORED PRODUCT INSECTS AND A STRATEGY TO PREVENT ITS INCREASE.

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The threat to the current availability of methyl bromide due to it being listed as an ozone-depleting substance under the UNEP Montreal Protocol means that there will be an increased use and mis-use of phosphine for the disinfection of stored products from insect pests. Phosphine resistance is a major problem in the control of some species due to inadequate attention to fumigation standards. The situation will only deteriorate unless a pro-active strategy for dealing with the resistance is implemented. In five stored product beetle species studied to date, the resistance is partly controlled by a major semi-recessive gene which controls uptake together with a modifier gene(s). For four species, a rapid resistance test based on the immobilisation of adults, rather than on mortality, has been developed which enables heterozygotes and homozygotes to be rapidly detected in populations before fumigation. The development of such tests is recommended for all species for rapid resistance diagnosis. They are also invaluable tools for the rapid generation of sufficient data for an accurate study of the genetics of the resistance in species where this is lacking. Heterozygotes can be controlled by modest increases in dose over those required for the control of susceptible insects. This should be achievable in carefully executed fumigations using current fumigation standards. Where the genes have already been selected, the doses for practical control should be re-defined from strains carefully selected in the laboratory to be homozygous for all the genes for resistance and fumigation standards adapted accordingly. The availability of information on the resistant genotypes present in a population coupled with a knowledge of accurately determined doses necessary to control all life stages of each genotype enables a decision to be made, before fumigation, on the appropriate dose to be applied taking the gas-tightness of the situation into account. These will prevent the selection of the resistance gene(s) or will eliminate homozygotes where they are produced. The careful application of these principles means that phosphine itself can be used to control resistant populations without producing unacceptable residues in commodities.

18-033

FUMIGANT PROPERTIES OF MOUNTAIN BIG SAGEBRUSH, *ARTEMISIA TRIDENTATA* NUTT. SSP. *VASEYANA* (RYDB) FOR STORED GRAIN INSECTS

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Vapors released by a patented process (Dunkel et al. 1994) that utilized mountain big sagebrush, *Artemisia tridentata* ssp. *vaseyana*, were hypothesized to have insecticidal properties similar to the fumigant methyl bromide. Bioassays with the lesser grain borer, *Rhyzopertha dominica* (F.), 0-3 days after adult emergence indicated an LT₅₀ of 6.93±1.16 hours (hrs) from the volatiles generated from only 0.029 g dry processed plant material per cc headspace. Hatching of eggs of the Indianmeal moth, *Plodia interpunctella* Huebner, was completely suppressed when exposed 4-20 hrs after oviposition to 0.0073 g processed plant material per cc headspace in a container that allowed passive diffusion and in which the terpenes disappeared by 48 hrs. Adult red flour beetles, *Tribolium castaneum* Herbst were also tested. Gas chromatography/mass spectrometry (GC/MS) analyses of the headspace above this processed plant material indicated in an airtight container, there were 7 detectable compounds. The two main components of the headspace were in a mean ratio of 1:3.2, initially, but over 24 hrs, gradually shifted to 1:2.4. The ug/cc headspace of each detectable compound in a sealed container was followed intensively for 72 hrs, and less frequently 120 days. Active compounds released by the plant material in a closed, but not airtight container, were not detectable after 24 hrs with GC/MS analysis of the headspace. Fumigative studies with the same ratio of the two main compounds generated synthetically indicated embryos of *P. interpunctella* and adults of *R. dominica* were as sensitive to the synthetic mixture as they were to the processed natural material. Although one could apply the precise commercial terpenes in the same ratio, the plant material provides a natural formulation that is automatically diluted (formulated) to levels safe for handling. Therefore, this preparation method with this plant material shows good potential as an alternative to methyl bromide in stored grain and commodity fumigations.

18-035

CONTROL OF THE IMMATURE STAGES OF *Sitophilus oryzae* (L.) SUSCEPTIBLE AND RESISTANT TO PHOSPHINE BY PHOSPHINE FUMIGATION.

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The objective of the present work is the determination of the dosages of phosphine required for the control of the immature stages of *Sitophilus oryzae* (L.) at 25°C and 60% R.H. A susceptible strain and resistant strains with different genetic backgrounds were tested. Cultures containing the naturally tolerant eggs and pupae of a susceptible reference strain a laboratory strain (476) selected to be homozygous for the major resistance gene and a resistant strain maintained in the laboratory without selection (Maringa, Brazil) were fumigated in laboratory chambers. Heterozygous resistant pupae produced from single pair crosses between the susceptible strain and strain 476 were also fumigated. The eggs of the susceptible strain were controlled by a 72 h exposure to a concentration-time product (CTP) of 36.55 ghm⁻³ whilst eggs of strains 476 and Maringa survived an exposure of 120 h to a CTP of 57.31 ghm⁻³ but not 168 h to a CTP of 75.87 ghm⁻³. For all strains tested the pupae were more tolerant than the eggs. Pupae of the susceptible strain were controlled by a 96 h exposure to a CTP of 47.38 ghm⁻³. Pupae of strain 476 survived an exposure of 120 h to a CTP of 57.31 ghm⁻³ but not 168 h to a CTP of 75.87 ghm⁻³ which allowed survival of pupae of strain Maringa. Pupae of strain Maringa were controlled by exposures of 168 and 240 h to CTPs of 106.44 and 100.87 ghm⁻³ respectively. The higher tolerance of some pupae of strain Maringa is of concern since it shows that selection in the field can produce a very resistant strain with possibly an enhanced resistance mechanism(s) or an additional mechanism(s). It is encouraging that the heterozygous resistant pupae were controlled by a 96 h exposure to a CTP of 47.88 ghm⁻³, a similar dose to that which controlled susceptible pupae. This indicates that the resistance genes can be eliminated when they are in the heterozygous condition and therefore, if continuous attention is given to the quality of fumigations, these genes can be eliminated when they are at a low frequency in the population.

18-036

THE POSSIBLE USE OF PLANT VOLATILES AS SUBSTITUTES FOR METHYL BROMIDE

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The fumigant toxicity of a large number of essential oils extracted from various spices and herb plants against five major stored-product insects was assessed. Eggs and pupae were found most resistant to the essential oils compared to adult and larvae. In the case of the most active compounds a concentration of 1.5 - 3 $\mu\text{l/l}$ air and exposure time of 24 h was enough to obtain 100% mortality of all adult insects tested. In columns filled with 70% wheat a higher concentration of around 50 and 70 $\mu\text{l/l}$ and a 7 - day and 4 - day exposure time respectively are needed to obtain 100% mortality of the adult insects tested. The rate of sorption and residues of the active compound in grain was also assessed. Studies to elucidate the effect of monoterpenes on the enzyme acetylcholinesterase in two stored-product insects showed that this enzyme is not the main site of action of these compounds.

18-038

INTERACTIONS OF MICROFLORA AND ARTHROPODS IN STORED GRAIN

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Mycologists and entomologists have separately studied the interaction of temperature and moisture on the colonization patterns of fungi and arthropods in stored grain. Knowledge of the secondary metabolites produced by fungal taxa common to grain stores can suggest sources of mycotoxin/entomotoxin contamination and point to volatile fungal metabolites that may attract or repel certain arthropods. Investigations of interactions between grainary arthropods and fungi should recognize the trophic relationships of species, initially focusing on fungi and arthropods that share the same optima for growth and reproduction. Sap beetles (*Carpophilus* spp.) can influence the initial fungal colonization of maize grain before it is harvested. Primary grain feeding insects damage intact grains providing routes of entry for fungi, secondary insects, and mites. The latter include detritivores and fungivores that selectively consume fungi, choosing among a mosaic of fungal 'patches.' Arthropod grazing can stimulate or suppress fungal sporulation, while some adult beetles such as *Tribolium* spp. produce benzoquinones which inhibit fungal growth. Detritivorous insects are better able to tolerate molded and mycotoxin-contaminated resources than are herbivorous insects. Fungivores and secondary feeders may benefit from fungal toxins 'antibiotics' that cure parasitic infections.

18-037

THE CURRENT STATE OF SELF-MARKING MODELS

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Self-marking models allow a mark-recapture type of population estimate from insect trapping. The estimates may be biased if certain model assumptions are violated. Violations of the original model are described by more complex models with larger numbers of parameters. Recent development has centered on model identification and selection, and alternative means for estimating the confidence interval. Results indicate that capture probabilities for more complex models are best determined numerically, using a general solution for the system of linear differential equations that describes self-marking and capture. Many alternative models can be identified in self-marking data, and likelihood-ratio tests do a reasonable job of selecting models. Finally, the bootstrap is now the best means of estimating a confidence interval. Bootstrap estimates allow us to incorporate model-uncertainty into the estimate, and perform significance tests using self-marking experiments.

18-039

COMPREHENDING STORED-GRAIN ECOSYSTEMS

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Understanding the dynamics of stored-grain ecosystems is essential to developing rational strategies for maintaining grain quality. Despite their apparent simplicity relative to many other ecosystems, stored-grain ecosystems are nevertheless quite complex, and their characteristics vary with geographic location, type of grain, and type of storage structure. Conceptual models developed over the past thirty years provide a useful framework for characterization and investigation of dynamics. Progress now depends upon our ability to identify, measure and analyze the key factors that drive these systems and then develop valid mathematical abstractions. The purpose of this workshop is to review progress in these areas.

18-040

THE IMPACT OF VARIABILITY IN LIFE HISTORY PARAMETERS ON MODELLING INSECT POPULATION GROWTH BASED ON DATA FOR *Sitotroga cerealella* IN STORED CORN

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A computer model is presented for the population dynamics of *Sitotroga cerealella* on stored corn. The model is used to test assumptions regarding the impact of variability in life history parameters across strains or biotypes of this species. The model is also used to evaluate the impact of the completeness of life history data collection on the accuracy of resulting models. Specific questions asked are: 1) How does life history data collected over a full range of conditions that support growth compare with data collected at more optimal temperatures only? and 2) Can extrapolation compensate for this missing data? The model assesses these questions for all population growth and development parameters including fecundity, fertility, and longevity of adults, as well as for immature development and survival. We also compare the resolution of these various forms of the model with our validation data to determine the significance of any predicted differences in terms of measured population growth.

18-042

SPATIAL DISTRIBUTION AND MOVEMENT OF INSECTS IN BULK WHEAT IN RESPONSE TO AERATION

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The study of the spatial distribution and movement of insects in bulk grain is technically very challenging, and historically has been done in small volumes of grain. The behaviour in large grain bulks with low density populations has remained an important but relatively unstudied issue. This paper describes a project that links large scale field studies with small scale laboratory studies to analyse the movement of insects in bulk grain that is subjected to cooling by aeration.

Using probe traps and equipment to set and retrieve these from depth, we tracked the movement of several species of beetles in a 6000 tonne aerated bulk of wheat and correlated these to the temperature gradients in the grain. Some species showed distinct responses to the temperature front, whereas others did not.

In order to investigate the spatial distribution and movement of the 5 most common species of stored product Coleoptera in Australia in detail, a laboratory apparatus was constructed that uses small enough amounts of grain and insects to be tractable, but which reflects the temperature and humidity relationships of a full-sized bulk. The apparatus consists of vertical columns of grain each comprising 10 sections that can be disassembled, plus all the equipment necessary to condition the input air.

Using this apparatus, beetles were exposed to upwards or downwards aeration, at 3 rates of air flow and to different gradients of temperature. From these experiments, the gravity response in non-aerated grain, the effect of aeration with warm air and of aeration with cooling air on the movement and distribution of beetles were determined and these were generally consistent with the field observations.

18-041

PRINCIPLES OF TRAP DESIGN FOR STORED-PRODUCT INSECTS

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To develop an effective pheromone baited trap for stored-product insects, several factors must be considered. These factors include the target species and the environment in which the trap will be used. An effective trap exploits behavior characteristics of the target species that may increase the likelihood of being trapped. Behavior patterns that might be considered are the insects tendency to climb or enter tight dark places. Environmental concerns include how dusty, moist, or the amount of space available for the trap. Other considerations are the ease of counting trapped insects, type of pheromone dispenser used, and the durability of the trap. To illustrate the development of a pheromone baited trap, the evolution of the FLIT-TRAK M² will be discussed. The FLIT-TRAK M² is a pitfall trap that has been successfully used to trap a variety of stored-product Coleoptera.

18-043

MODELLING POPULATION DYNAMICS OF THE MAIZE WEEVIL *SITOPHILUS ZEAMAI* MOTSCHULSKY (COLEOPTERA: CURCULIONIDAE) AND ITS HYMENOPTERAN PARASITOID *ANISOPTEROMALUS CALANDRAE* (HOWARD) (HYMENOPTERA: PTEROMALIDAE)

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A temperature-driven age-structured computer simulation model was developed to help evaluate different strategies for augmentative biological control of the maize weevil *Sitophilus zeamais* in stored maize by the parasitic wasp, *Anisopteromalus calandrae*. Parameters of the model were based on detailed laboratory life history and functional response studies conducted at 20, 25, 30 and 35°C.

Establishment of a sufficiently large parasitoid population during the first host generation is critical to the success of biological control in this system. Simulation was used to explore the efficacy of various release strategies including time of first parasitoid release, numbers per release, time between consecutive releases, and number of consecutive releases. An optimal strategy at 25°C includes two or three sequential releases, using about ten times as many parasitoids as adult weevils, at 9 day intervals as soon as the first hosts of suitable size (≥ 15 days old) are available.

Temperature affects population growth rate of the two species differently. Growth rate of the parasitoid is greater than that of the weevil above 21°C, and this difference increases dramatically above 29°C. This indicates that the parasitoid may be particularly useful for warm storage conditions.

18-044

MATHEMATICAL MODELS FOR
STORED-GRAIN ECOSYSTEMSD.S. Jayas¹, W.E. Muir¹, N.D.G. White²,
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Mathematical models of heat, moisture, and gas transfer through bulk grains can be used as tools for designing storage structures and for monitoring the stored commodity. The mathematical models published in the literature will be reviewed and evaluated. The approach to develop and validate mathematical models will be discussed. The need for measuring and reporting various parameters needed in the development of mathematical models will be outlined. Using the models developed by our group as examples, the utility of the models will be discussed.

18-046

THE ROLE OF GRAIN LIPIDS IN STORED
PRODUCT INSECT LIFEZ. Winiecki¹, J. Nawrot¹, J. Szafraniek²,
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The main function of grain and insect surface lipids is to minimise the losses of body water through the cuticle. In plants and their seeds the lipid layer is the first element that the insect comes in contact during searching for food or place for egg-laying. The results of investigation on another properties of grain cuticular lipids in food recovery and food utilisation by storage insects and differences in chemical composition of lipids in development stages and different sexes of insects are also presented. The composition of substances found in cuticular lipids of grain and grain feeding insects is very diversified. Only n-alkanes occurred in all examined insect species and wheat grain showed the high correlation in the proportion of main components.

18-045

CHEMOSENSORY AND TACTILE REGULATION OF COPULATION
AND OVIPOSITION BY *CALLOSOBRUCHUS CHINENSIS* L.
AND *C. MACULATUS* FABRICIUS (COLEOPTERA, BRUCHIDAE)I. Yamamoto, H. Honda, T. Miyamoto, K. Ohsawa¹
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C. chinensis deposits eggs not only on beans but also on surface of certain curvature, while *C. maculatus* oviposits mainly by responding to chemical stimuli in the seed coat. There are two prominent phenomena that are associated with the oviposition behavior of both weevils. Under low population density conditions, the females oviposit evenly among beans, being guided by a marking pheromone. Under high density conditions, the number of eggs on each bean becomes high and egg distribution becomes random, but only a few eggs can hatch and grow normally inside a bean, leaving the remaining eggs to die by the ovidical action of higher doses of the marking pheromone. These weevils thus have developed a strategy to reduce competition among larvae and to maximally utilize the host beans by using the same substance at different levels. The two weevils can grow in many beans, but not in kidney bean due to the growth inhibitor in the bean. Immediately after emerging from beans, the adults make copulation. The sex attractant released from the female attracts the nearby male. When they are in close proximity, the second sex pheromone having no attractancy functions to induce erection and insertion of male genital organ and ejaculation. The ecochemicals involved in the above behaviors were partially or completely identified from the two weevils. They have some application for the pest control.

18-047

ANALYSIS OF SPECIFIC PROTEINS OF SYMBIOSIS IN THE WEEVIL *SITOPHILUS*

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The weevil *Sitophilus* harbours intracellular symbiotic bacteria into specialized cells (bacteriocytes), which interfere with the host metabolism and supply the weevil with 5 vitamins. As a consequence, these bacteria increase the fertility and the rate of development of their host and are therefore responsible of the harmfulness of *Sitophilus* against stored cereals. Despite the fact that the symbiotic bacteria are highly integrated to the insect physiology, symbiosis can be disrupted by heat treatment and aposymbiotic weevils can be obtained. On a more fundamental point of view, this model allows us to study genetic interactive factors that are needed to maintain the symbiotic association.

Pulse field electrophoresis experiments have shown that the symbiotic bacteria chromosome is about half smaller than that of *E. coli* (the closest free living bacteria), indicating that some prokaryotic genes have probably been deleted or exported to the nucleus of the bacteriocytes during the coevolution of both partners.

We have also compared the protein patterns of two strains of *Sitophilus oryzae* (symbiotic and aposymbiotic) in order to detect some proteins synthesized by the host in response to the presence of the endocytobiont. Among the five detected proteins, we paid more attention on one (60kDa, pI=6) which belongs to the chaperonin family. This protein was called symbionin, the term being firstly used in 1982 by Ishikawa about the endocytobiosis of the aphid *Acyrtosiphon pisum*.

The endocytobionts synthesize more than one hundred proteins *in vitro*, and preferentially three of them *in vivo*. Western and northern blot analysis show that one of these proteins is the symbionin. The gene encoding the symbionin have been cloned and sequenced.

Chaperonins are ubiquitous proteins found in all cellular types, especially in parasitic bacteria. Considering that endocytobionts could have evolved from parasitic bacteria, it is thus not very surprising to find that they contain a chaperonin. But the most interesting questions are to know why this chaperonin is produced in such a great quantity by the endocytobionts, and whether the presence of symbionin is a general process of endocytobiosis conserved during evolution. The most conceivable hypothesis for the biological role of symbionin is that it might be produced for the endocytobiont own safe, and could act as a host protein pumping and assembling machinery.

18-048

COCKROACH NUTRITION: A GUIDE TO BETTER PEST CONTROL

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Nutrient intake is critical for development, growth, and reproduction of the German cockroach, *Blattella germanica*. Development of nymphs is tightly linked to availability of food and its quality. Adult females are also highly dependent on food during each gonotrophic cycle. At each stadium and in the adult female, a cycle of feeding corresponds to specific physiological events. In nymphs and adults, the synthesis of cuticular lipids is linked to availability of food and in adult females, juvenile hormone biosynthesis is linked to a gonadotrophic cycle. Clearly, these links are central to strategies and tactics that rely on insecticidal baits for cockroach control.

Nutrient characteristics of chemically defined baits ("bait base") significantly affect rates of food intake by different life stages of the German cockroach. Moreover, the nutrient status of feral cockroaches affects the amount of food removed from such baits in homes. Coprophagy plays an important role in vectoring active ingredient to non-feeding life stages and the nutrient characteristics of baits can affect the efficacy of their secondary effects. Strategies for targeting non-feeding stages with insecticidal baits will also be discussed.

18-050

INSECT SPECIES OCCURRING ON DATE PALM TREES AND STORED DATES IN THE SIWA OASIS, EGYPT

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Field investigation and sample identification during the period from July 1994 until June 1995 were carried out in orchards of different regions of the Siwa Oasis. The occurrence of 18 insect species was recorded on date palm trees and/or date fruits.

Among the most injurious insect pests found, were *Cadra calidella* (Guen.), *Myelois ceratoniae* (Zeller), *Arenipses sabella* (Hamps.) and *Parlatoria blanchardi* (Targ.). Two hymenopterous parasitoids, i.e. *Phanerotoma flantestacea* (Fisher) and *Bracon hebetor* (Say) are common on the larvae of date moths.

The results include records of infestation levels by pest species on growing date palms and stored date fruits. Percentage of infestation found on dried fruits in open air storage were calculated. The fruits of different varieties of palm trees differ widely in their rate of infestation. The highest rate of infestation was recorded for the variety saeydi.

The occurrence of both date fruit moths *C. calidella* and *M. ceratoniae* was recorded for the first time on fruits of the Napk tree (*Ziziphus* sp.).

18-049

ASSESSMENT OF THE INHIBITORY EFFECTS OF BIOLOGICALLY ORIGINATED SUBSTANCES ON THE DEVELOPMENT OF THE MAIZE WEEVIL, *SITOPHILUS ZEAMAI* MOTCH. (COLEOPTERA : CURCULIONIDAE)

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The recent progress in plant transgenic technology, make it possible, in the near future to introduce a new variety of rice resistant to *Sitophilus zeamais*, an insect most destructive to stored rice. However, basic knowledge of genetically regulated substances having inhibitory or regulatory effects on growth and development of this insect is still lacking. We developed an artificial diet method to evaluate the inhibitory action of biologically originated substances or materials for the weevil. In the search for such compounds, we found that soybean showed the strongest inhibitory action on the growth and development of *S. zeamais* among the materials tested, such as "oryzacystatin", a proteinase inhibitor of rice and "CB1", a chitin binding inhibitor obtained from *Bacillus licheniformis* and also a number of legumes. Our studies also show that "saponins" found in a concentration of about 0.5% in soybean play an important role in the inhibition of development of immature stages of *S. zeamais*.

18-051

INFESTATION OF TWO DATE-FRUIT VARIETIES WITH SOME NITIDULID BEETLES (COLEOPTERA: NITIDULIDAE).

BY

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ABSTRACT

A study of the infestation of two date-fruit varieties with the nitidulid beetles, *Carpophilus humeralis*(F.), *C. dimidiatus*(F.), *C. hemipterus*(L.) and *Haptoncus luteolus*(Erich.) was carried out from August 1994 till January 1995. This investigation was fulfilled on the fallen fruits of Zaghloul and Semmany dates in the North Eastern Coast of Delta of Egypt.

The results indicated that the degree of infestation in summer was generally higher with *Carpophilus hemipterus* than the other nitidulid beetles .

Also, the degree of infestation of Zaghloul and Semmany in winter with the dried-fruit beetles was generally in high rate , except for *carpophilus hemipterus* which was more abundant on Zaghloul variety. The results also showed that the degree of infestation of Zaghloul and Semmany with *Haptoncus luteolus* and *Carpophilus humeralis* was higher in winter than in summer.

18-052

Chemistry of Methyl Neodecanamide (MNDA) - A New Insect Repellent**R. J. Steltenkamp¹****Colgate-Palmolive Company, 909 River Road,
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Methyl Neodecanamide (MNDA) is a new chemical that is highly effective in repelling a variety of household pests including cockroaches and ants. MNDA is a member of a class of branched secondary neoalkanamides that are most effective within a specific molecular weight range with a total carbon number between 11 and 14.

MNDA is a safe compound that has strong affinity to surfaces and has excellent stability and aesthetics. As a result, MNDA can be incorporated into a home cleaning product where it can provide benefits as a repellent of nuisance pests having public health importance.

18-054

An Evaluation of MNDA Repellency to Household Pests Found in India**T. N. Ananthakrishnan¹****Entomology Research Institute Loyola College,
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Evaluation of MNDA repellency against native Indian household pests has shown its significance as a useful repellent. Test protocols were developed to assess repellency to a wide variety of native pests. These pests included numerous species of ants, silverfish, beetles and local species of cockroaches. MNDA has been shown to be a more effective repellent than the standard Diethyltoluamide (DEET) in these protocols. These test protocols and repellency results will be presented along with their significance in controlling household pests.

18-053

Insect repellent effects of alkyl neoalkanamides: A new tool for indoor IPM**Coby Schal¹****Department of Entomology****Box 7613****North Carolina State University****Raleigh, NC, USA-1**

Control of pests in structures relies heavily on habitat modification, insecticides, and traps. Physical modification of the indoor environment, including sanitation and resource elimination, usually enhances the effects of other control measures against cockroaches. By reducing limiting resources for population growth and by facilitating movement of insects, physical changes can increase the probability that pests will contact residual insecticides and they can reduce areas which otherwise would require insecticide treatment. Repellents can be used in efforts to alter existing structures in order to reduce these resources. This paper will review data on the efficacy of new secondary amides as cockroach repellents, including structure activity relationships, dose-response studies, results with other cockroaches, repellency to other insect species, and comparisons with other repellents. These secondary neoalkanamides may constitute a new tool in reduced-risk integrated pest management of indoor pests.

18-055

Measuring Spatial Displacement of German Cockroach Populations Pressured by Repellent-treated Harborage**Dr. Richard J. Brenner¹****USDA, Agricultural Research Service, Medical &
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The use of a cockroach repellent as a tool in integrated pest management strategies would represent a novel and significant departure from traditional reliance on toxicants. The research and development process requires radically different analysis techniques; whereas toxicants impact mortality, repellents impact spatial distributions. This paper describes spatial statistical analysis techniques developed to measure the potential of N-Methyl Neodecanamide (MNDA) to manipulate cockroach populations. The technique was used to document dramatic shifts in population distribution following treatment with 2% MNDA of 50% of cockroach harborage that cumulatively encompassed 85% of the population. Subsequent research in specially constructed and electronically monitored environments is focusing on the repellent's effectiveness (1) in preventing infestation of selected areas, and (2) in "herding" pests toward strategically placed toxic baits. Proposed field trials in cooperation with the US. Department of Defense and the US. Environmental Protection Agency will be discussed in which this repellent will be used in a reduced-risk integrated pest management program.

18-056

Surfactant Based Delivery System for the Insect Repellent N-Methyl Neodecanamide (MNDA)

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Solutions of MNDA used to wash surfaces were found by extraction and analysis to deposit MNDA at efficacious levels. The strategy of modifying and “manipulating” insect behavior is promising. Solutions containing MNDA could be used to render areas “inhospitable”, and “move” insects away from indoor areas where insecticide use is discouraged. In conjunction with judicious use of appropriate insecticides, repellents solubilized in a surfactant base could also be used in an integrated approach to manage insect populations by providing sanitation and creating “pest exclusion zones”; thereby increasing the frequency and duration that insects are in contact with insecticides. This paper will review clinical studies done in homes to determine the efficacy of MNDA containing surfactant solutions against household pests such as cockroaches and ants.

18-058

AGGREGATION PHEROMONES OF WEEVILS ASSOCIATED WITH STORED PRODUCTS IN THE GENUS SITOPHILUS - THEORETICAL AND APPLIED ASPECTS

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The maize weevil, the rice weevil and the granary weevil are closely related species in the genus *Sitophilus*. All are associated with stored grain of all kinds and cause severe damage in post harvest systems worldwide. Sitophilone the aggregation pheromone of *Sitophilus zeamais* and *S. oryzae* as well as sitophilate of *S. granarius* have been identified. Behavior studies on *S. zeamais* and *S. oryzae* towards natural and synthetic pheromone sources will be reviewed and compared to recent studies on *S. granarius*. Orientation towards the pheromone can best be described as orthokinesis where weevils of both sexes show arrestant behavior near the odor sources. This might lead to a modified trapping strategy combining baited and unbaited traps.

18-057

APPLYING PHEROMONES IN STORED PRODUCT PROTECTION - A REVIEW AND PROSPECTIVE NEEDS

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Most pheromone components of post harvest pest arthropods of major economic importance have been identified successfully during the past 25 years. Many of the pheromones are available for both lepidopteran and beetle species. Sex and aggregation pheromones for beetles and weevils are often multiple compound complexes with optically active enantiomers, which physical characters are often very similar but their physiological effects are generally different.

Twenty-five years of applied pheromone research and field data will be reviewed.

This will include successful monitoring of pest insects and proper timing of control, mass trapping and the strategy of attract and kill. The technique of mating disruption will be discussed.

Pheromones act as attractants, arrestants or repellents, thus regulating population dynamics. Their successful application depends on the optimal concentration, suitable dispensers and effective trap design. Pheromones are generally regarded as intraspecific active semiochemicals, but they very often also function interspecifically as allomones or kairomones. In biological pest control kairomones are becoming more important for monitoring the presence and abundance of predators and parasitoids.

The identification of a pheromone's chemical structure can only be the beginning in understanding an odor based communication system.

While much has been learned in field trapping research, many questions remain. Some of them will be addressed.

18-059

SEX PHEROMONE BIOLOGY OF *CALLOSOBRUCHUS SUBINNOTATUS* AND *C. MACULATUS*: SITES OF PRODUCTION, CHEMISTRY, AND PHYSIOLOGICAL AND BEHAVIORAL RESPONSES

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This paper discusses several aspects of the sex pheromone biology of *Callosobruchus maculatus* and *C. subinnotatus*, including ultrastructure, chemistry, behavior, and electrophysiological responses. Type 3 secretory cells on pygidium of females have been localized as the source of the sex pheromone in these two species. These cells open to the exterior via cuticular pores, which appear to be more numerous on the pygidium. Secretory cells and pores are present in both females and males; however, we have not been able to ascribe a function to the male structures. Organic solvent extracts and entrained volatiles from females only elicit behavioral and electroantennographic (EAG) responses from males. Both species appear to utilize mixtures of volatile, short chain fatty acids as sex pheromone, characteristic of several species of bruchids. *C. maculatus* are sexually active throughout photophase, but virgin females entrained to a 16:8 L:D light cycle emit more sex pheromone during the first half of photophase than during the second half of photophase or during scotophase. Sex pheromone production is affected by age and mating status, but is not affected by the presence of oviposition hosts. Males respond with upwind orientation to and attempt to copulate with substrates containing pheromone. The potential use of sex pheromones and other behavior modifying substances in the management of bruchids will also be discussed.

18-060

PHYSICAL CONSTRAINTS ON PHEROMONE DISPERSAL IN STORAGE ENVIRONMENTS: EFFECTS ON ORIENTATION BEHAVIOR

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Orientation of flying insects to pheromone in a closed, warehouse environment is not as efficient as that in a field environment where wind direction and plume intermittency provide additional cues about the location of a pheromone source. The effects of adsorption and re-emission of pheromone from nearby surfaces also are relatively more important in a storage than in a field environment. In this report, the alterations in searching behavior that result from such constraints are considered by three dimensional analysis of searching behavior flights by male *Cadra cautella* (Lepidoptera: Pyralidae). The shape of the plume around a pheromone-emitting female is inferred from comparisons with published descriptions of flights in a wind tunnel.

18-062

BEHAVIORAL AND PHYSIOLOGICAL MECHANISMS UNDERLYING MATING DISRUPTION IN ALMOND MOTHS USING A NOVEL SEMIOCHEMICAL RELEASE DEVICE

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A novel, truly controlled release system for long term storage and intermittent delivery of semiochemicals over long periods of time, at specified release rates, was developed and tested for use in the field as mating disruptant or attract-and-kill bait for *Cadra cautella* (Walker) (Lepidoptera: Phycitinae). There were no signs of degradation or reduction of biological activity of pheromone stored in the aerosol canisters of timed, metered, semiochemical release (TMSR) devices that were kept at ambient temperatures in the field throughout the summer. Pheromone released upon demand from those cans disrupted all mating of freely flying virgin *C. cautella* males and females at a 2:1 ratio kept for 24 h in storage rooms, and up to 92% mating for 72 h. Mating disruption was measured directly by examination of the bursa copulatrix of recaptured females for spermatophores, indicators of successful mating encounters. TMSR devices delivering 50 µg pheromone spray⁻¹ 15 min⁻¹ disrupted mating by more than 90%, independent of either moth population density or of number of TMSR devices used per room. TMSR devices delivering 5 µg spray⁻¹ 15 min⁻¹ disrupted at least 60% of mating. Addition of 1% natural pyrethrin extract to the 5 µg pheromone sprays enhanced disruption (to more than 80%) by exterminating 96% of the male population in 24 h. Observation of the moths in the field experiments, and complementary laboratory experimentation, indicates that elevation of pheromone response threshold of males pre-exposed to pheromone from TMSR devices plays a major part in disruption of *C. cautella*. The antennae of pre-exposed males produced smaller electroantennographic responses to pheromone than did the antennae from naive males. Furthermore, competition between synthetic sources and calling females, as well as false trail following were implicated in our observations as mechanisms that enhance mating disruption of *C. cautella*. We will discuss the new applications (and possible limitations) of the use of TMSR devices to deliver semiochemicals for the management and control of storage and urban insect pests.

18-061

EFFECTS OF PHEROMONE PLUME STRUCTURE ON UPWIND FLIGHT AND CASTING RESPONSES OF THE ALMOND MOTH

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The upwind flight of male moths to conspecific females is mediated by the chemical and structural characteristics of a pheromone plume. The reaction of male *Cadra cautella*, the almond moth, to the brief interception of single pulses of sex-pheromone, the smallest structural units of odour plumes, is to interrupt casting flight by suppressing counterturning and briefly adopting a straighter upwind heading before reverting to casting flight. These behavioral reactions to pheromone contact and loss support the phasic-tonic model of odour-modulated flight, in which an underlying tonic counterturning rhythm, expressed upon pheromone loss, is briefly overridden by phasic upwind surges, expressed upon pheromone filament interception. *C. cautella* males fly increasingly faster and straighter as the duration of pheromone pulses, mechanically generated at a constant frequency of 2Hz, increases from 20 to 300 ms, culminating with the highest levels of such flight at 400 ms and continuously generated plumes. Surprisingly, the shape and form of the flight tracks of males exhibiting casting flight following the loss of pheromone likewise was correlated with the structural differences of the odour plumes in which males had recently flown. Moths that had flown upwind fast, in nearly straight tracks along rapidly-pulsed or continuous plumes, exhibited downwind casting flight soon after pheromone removal. On the other hand, males that had flown upwind slowly in wide zigzagging tracks in response to slowly-pulsed plumes exhibited long, stationary zigzag flights during casting in clean air following plume loss. Such males took three times as long to drift downwind away from the field of view than those that had flown upwind in response to either rapidly-pulsed or continuous plumes. The after-effects of contact with plumes of various structures are thus long-lasting and may indicate that the pheromone-mediated programs of anemotaxis and counterturning are constructed to respond to either rapidly or slowly shifting air-currents and wind-fields.

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18-063

ENDOGENOUS REGULATION OF PHEROMONE BIOSYNTHESIS IN *Plodia interpunctella*

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Plodia interpunctella female moths attract conspecific males by emitting pheromone during a defined period of the night. The main pheromone component was identified as Z,E, 9, 12 tetradecadienyl acetate (ZETA). In this study the mechanism of control of pheromone production was studied¹. Brain-subesophageal (Br-SOG) extracts of *P. interpunctella* possessed pheromonotropic activity when tested on the pheromone production of *Helicoverpa armigera* females. A significant stimulation of pheromone production by *H. armigera* females was observed when they were injected with 1 Br-SOG equivalent from *P. interpunctella* as well as the related moth *Ephestia cautella*. Decapitation of *P. interpunctella* females for 24 hours prevented normal production of ZETA. This was reversed by injections of synthetic pheromone biosynthesis activating neuropeptide (Hez-PBAN) as well as Br-SOG extracts. In addition, Hez-PBAN, injected to *P. interpunctella* during the photophase induced a large increase in ZETA production. Mating caused a sharp decline 3 hours afterwards. Using isolated pheromone glands, Hez-PBAN was also shown to stimulate pheromone production *de novo*. This stimulation was significantly inhibited by the neurotransmitter, octopamine, in a dose-dependent manner thereby implicating its modulatory role in pheromone production as was observed in *H. armigera*².

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18-064

SOME ASPECTS OF THE SEXUAL BEHAVIOUR OF THE LEPIDOPTERA PYRALIDAE INFESTING STORED-PRODUCTS

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Research on moth sexual behaviour has focused primarily on female pheromones and male behavioural responses to them. Although several researches on moth sex pheromones have demonstrated that the complete sequence of courtship behaviour also involves male pheromones and other types of communication, relatively little work has been done on the connection between pheromones and extrusible organs or with sound production.

Many insects have airborne sound receptors, which function primarily in intraspecific communication whilst in others hearing serves to warn of the potential threat of a predator.

Certain pyralid moths infesting stored food, such as the Galleriinae *Achroia grisella* F., *Galleria mellonella* L. and *Corcyra cephalonica* (Staint.), and the Phycitinae *Ephestia cautella* (Walker), *Ephestia kuehniella* Zeller and *Plodia interpunctella* (Hübner) have acquired the additional ability to generate sounds by wing-fanning for intraspecific communication and pair forming.

The courtship of these Lepidoptera Pyralidae has been examined and several studies have been made on the male orientation to the pheromone produced by the calling female and also on the behaviour of the male after the female has been located.

The paper gives details on the role of ultrasound production in the courtship behaviour and discusses the role of other signals in pair forming.

18-066

SURVEILLANCE OF MOTH SPECIES (PHYCITINAE) ON STORED DATES IN MOROCCO BY MEANS OF PHEROMONE TRAPS

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In an attempt to monitor the moth species (Phycitidae, Phycitinae) infesting stored dates, adhesive pheromone-baited (TDA) traps (Detia Ltd., Germany) were set up in a store comprising dates of the variety "Khalt" in Zagora (southern Morocco) from February through August 1992. Four species of Phycitinae were trapped: *Cadra cautella* (Walker), *C. calidella* (Guénéé), *Plodia interpunctella* (Huebner) and *Ectomyelois ceratoniae* (Zeller). Those species represent 22.2, 35.8, 38.3 and 3.6 % of 720 moths caught on pheromone traps, respectively. The pheromone traps caught 77.5 % of the total catch, being males only. The unbaited adhesive traps (control) merely caught 22.5 % of the total catch, i.e. 97 male and 65 female moths. There was a close correlation between moth trapping and ambient temperature (ranging from 15 to 36°C).

E. ceratoniae was more common in the field than indoors, whereas *C. cautella*, *C. calidella* and *P. interpunctella* were mainly caught indoors. Three successive generations of the latter occurred between March and July.

Observations made on laboratory cultures of the above species revealed a close correlation between the time of moth flight and onset of infestation, i.e. oviposition on stored dates. Appropriate periods of date protection against phycitid infestation are thus end of March, first decade of May and /or end of June, in accordance with the trapping data.

Since the consumer rejects any infested date, economic loss is equivalent to the infestation rate, regardless of the number of larvae per date.

18-065

PHEROMONES OF STORED-PRODUCT INSECTS AND THEIR POTENTIAL IN PEST MANAGEMENT

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The use of pheromones, is one of several modern techniques that show promise in controlling stored-product insects, their utilization may lead to a drastic reduction of chemical treatments with consequent economic and qualitative advantages. Goods may be protected from possible pesticide residues and thus improve the image of the company's products.

Considerable progress has been made in recent years in monitoring and control of stored-product insects by using mass trapping, mating disruption and attracticide (lure and kill) methods.

Good results can be obtained by the use of the pheromones together with careful cleaning of the rooms, particularly in the corners and, above all, inside machinery. This procedure eliminates the possibility of insect reproduction in areas where food is present.

In the case of complex infestations, where more than one species of moth or beetle is involved, treatments which use only pheromones become complex and are not effective. Integrated treatments which use different technical means, not excluding chemicals achieve better results.

An important role should be assigned to all prevention techniques which effectively keep infested goods out of food-processing plants, mills, storehouses, etc..

18-067

EICOSANOIDS AS MEDIATORS OF CRITICAL PHYSIOLOGICAL FUNCTIONS IN INSECTS

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Eicosanoids are oxygenated metabolites of 20:4n-6 and two other C20 polyunsaturated fatty acids (PUFAs). These compounds are crucial mediators of many cellular events in humans and other vertebrates; their significance in insects and other invertebrates is now gaining increased recognition. Eicosanoids exert important actions in reproductive and thermoregulatory behavior of some insects. They also mediate cellular immune reactions and water and ion transport physiology in all insects so studied. Of particular interest in this symposium, we have identified two crucial eicosanoid actions in stored grain beetles.

First, eicosanoids mediate nodule formation in response to bacterial infections in larvae of the tenebrionid beetle, *Zophobas atratus*. Nodule formation is the major cellular immune reaction to bacterial infection in insects. We found that treating the insects with eicosanoid biosynthesis inhibitors prior to infections impaired the insects' ability to form nodules. Nodulation could be restored by subsequent treatment with eicosanoid-precursor PUFAs.

Second, we detected these PUFAs and prostaglandin E₂ in Malpighian tubules of the mealworm beetle, *Tenebrio molitor*. Based on findings in mosquitoes, prostaglandin E₂ probably regulates Malpighian tubule function in *T. molitor*. These results open wide the possibility of developing novel and insect-specific inhibitors that can impact insects during crucial stages of their life cycle.

18-068

JUVENILE HORMONE IN COCKROACHES: A MULTI-FUNCTIONAL TARGET SITE IN PEST CONTROL

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Juvenile hormone analogs (JHAs) have proven to be effective materials for cockroach control. They disrupt development by interfering with the transformation of the last instar nymphs to adults, resulting in sterile adults. In addition to governing the molt cycle, JH also plays a central role in female reproduction, including vitellogenin synthesis and uptake by oocytes, pheromone production, sexual receptivity, ootheca production, and the pattern of feeding. Using *Blattella germanica*, we have studied environmental signals that affect corpora allata (CA) activity, including social interactions and food quality and quantity. Internal physiological factors, such as the ovaries and signals associated with copulation also affect JH synthesis by the CA. Pregnancy in *Blattella* requires a period of low JH titer. We reasoned that application of high levels of JHAs might increase the frequency of abortions in gravid females. Removal of feeding inhibition, caused by removal of the ootheca, together with high levels of JHA, should further stimulate the previously gravid female to feed. We thus hypothesized that this strategy might increase the fraction of feeding females in a cockroach population, and therefore should increase the efficacy of insecticidal baits.

This novel strategy was tested both in the laboratory and in the field (120 homes). JHAs effectively enhanced the efficacy of insecticidal baits in both situations. As the rate of development of new insecticides declines, enhancing the efficacy of available insecticides will become an important tactic in urban pest management. Research into the basic biology (ecology, physiology, behavior) of pest species will guide such new strategies.

18-070

DISTRIBUTION OF *DERMATOPHAGOIDES* MITE ANTIGENS IN HOMES AND PUBLIC FACILITIES IN JAPANE. Konishi¹, K. Uehara^{1,2}

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Study on contamination of *Dermatophagoides* mites in human environment is important for prevention of sensitization and therapy of mite allergic patients. In order to show the current hygienic situation in Japan, we have surveyed distribution of mites in homes of allergic patients and non-allergic people, as well as public facilities, such as hospitals, hotels, ryokans, offices and movie theaters. Dust samples were collected mainly from floors and bedclothes, and examined for mite antigen levels by an enzyme-linked immunosorbent assay. The antigen detected by this method is characterized as a protein with a molecular weight of 180 kDa and distributed in most parts of the mite body. The results of survey among homes indicated that patient homes had higher antigen levels than control homes in comforters and pillows but not in futons, carpets, tatamis, and wooden floors. Samples with antigen levels of ≥ 10 ug/m² were more frequent in and around summer than other seasons of the year in most materials. Wooden structures showed higher antigen levels than concrete structures in comforters, pillows, carpets and tatamis in patient homes. The results of survey among public facilities indicated that clinically important antigen levels of ≥ 50 ug/m² were detected in 71% of samples from seats in a movie theater and 42% of samples from tatamis of guest rooms in two ryokans, whereas such a serious mite contamination was not observed in samples from offices and a hotel. Sickrooms of hospitals had antigen levels of ≥ 50 ug/m² in 2% of mattresses and 6% of painted and tiled floors but not in comforters, futons and pillows. Different antigen levels in these facilities suggested that low humidity conditions, regular cleaning of floors, and regular exchange of sheets or covers for bedclothes are effective to keep mite levels low.

18-069

CROSS-ALLERGENICITY OF THE HOUSE DUST MITES *EUROGLYPHUS MAYNEI* AND *BLOMIA TROPICALIS*L.G. Arlian, M.S. Morgan, E. Fernandez-Caldas¹Department of Biological Sciences, Wright State University, Dayton, OH USA and ¹CBF Leti S.A., Madrid, Spain

The domestic mites *Euroglyphus maynei* (EM) and *Blomia tropicalis* (BT) frequently co-inhabit homes in subtropical/tropical regions around the world. Both species are the source of substances that cause allergic reactions in patients. The purpose of this study was to examine the immunologic cross-reactivity between EM and BT.

Sera of 19 mite-sensitive patients who were skin test positive to BT and/or RAST positive to EM were used to probe immunoblots or crossed radioimmuno-electrophoresis (CRIE) gels. Western blotting showed that individual sera had IgE that bound to 0-17 and 2-15 proteins in EM and BT extracts, respectively. Corresponding IgE-binding proteins of 105, 75, 57, 18 and 14 kD were detected in both EM and BT extracts. The majority of IgE-binding proteins did not show corresponding bands in both extracts. Heterologous CRIE showed IgE binding to 6 of the 9 EM antigens precipitated by anti-BT serum with individual sera recognizing 0-4 of the 6 allergens. In the reciprocal reaction, 10 of the 12 proteins of BT that were precipitated by anti-EM serum bound IgE with individual sera binding to 0-5 proteins.

This study indicated that EM and BT are the source of both species-specific and cross-reactive allergens, but most allergens in each extract were species-specific.

18-071

BRONCHIAL ASTHMA DUE TO SENSITISATION TO STORAGE MITES.

RESULTS OF A MULTICENTER STUDY IN GERMANY

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This study describes clinical and allergological data from 90 patients, living in different regions of Germany. They were asthmatics, with relevant allergy to storage mites and / or house dust mites. Diagnosis based on clinical and allergological examinations, including bronchial challenge tests. Environmental conditions (at work places and in the homes) are described in detail. A total of 151 bronchial challenge tests were performed. The result is as follows

Allergen	Total no of pat.	No of positive	No of negative results.
Lepidoglyphus	43	31	12
Acarus siro	38	16	22
Tyrophagus	24	12	12
D. pteron.	46	22	24

The overall prevalence of allergy to storage mites in the general population is calculated with 5%, this is half of the estimated prevalence to house dust mites. 42 from the total of 90 patients were farmers, 5 patients worked with grain or flour, 7 with woods or with paper dust. 36 had not such work place. Thus, allergies to storage mites are also encountered in the „normal population“.

18-072

DENSITIES OF *LEPIDOGLYPHUS DESTRUCTOR* AND LEVELS OF ITS MAJOR ALLERGEN Lep d 1 IN GRAIN AND FLOUR

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Allergic Danish bakers display positive reactions in skin prick tests to several stored product pests even though no correlation to the development of allergic symptoms could be determined. The possibility of developing serious allergies following exposure to stored product pests is considered a potential risk to the health of workers involved in grain and flour handling.
The correlation between the presence of stored product pests and levels of a major pest allergen in samples of grain (wheat) and flour for human consumption was investigated. 1600 samples were collected from grain stores after different lengths of storage and from flour mills before and after the milling process. Extent of pest infestation was determined. The levels of the major allergen Lep d 1 deriving from the grain mite *Lepidoglyphus destructor* (Acarina: Glycyphagidae) was determined by means of ELISA; antibodies were supplied by Dr Ventas and Dr Polo, ALK-ABELLÓ, Spain.
In laboratory samples with mites added, the correlation between the mite density and the Lep d 1 level was good. Densities of *Lepidoglyphus destructor* were generally low in the collected samples, possibly due to warm and dry weather during harvest in 1994 and 1995. As a consequence, the Lep d 1 levels were also low, in most cases below the background level. Other mites species were more abundant; allergen levels deriving from these pests will be determined, if possible.

18-074

OCCUPATIONAL ALLERGIC PATHOLOGY INDUCED BY *TETRANYCHUS URTICAE* IN FARM WORKERS.

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Over the last 3 years a seasonal, occupational syndrome has been observed in a group of farm workers featuring rhinitis coupled mostly with bronchial asthma and urticaria or recurrent dermatitis on the hands (1,2). Since this syndrome had remained "undetermined" after standard and usual specific professional allergic diagnostic trial, the possible etiologic role of *Tetranychus Urticae* (TU), a macroscopic mite ubiquitous to warm climate commonly known as "red spider", has been investigated. Skin prick test, serum IgE dosage and different types of epicutaneous tests were performed with a TU-whole body extract prepared in our laboratory. This specific diagnostic trial allowed a definite diagnosis of TU-induced allergic or immune-mediated disease in 43 out of the 56 patients examined. Moreover, an epidemiological pilot study on unselected farm workers has shown a 2.5% cumulative prevalence of TU-induced sensitization and a 1.0% prevalence of TU-induced immune-mediated diseases.
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18-073

DOMESTIC MITES AS A CAUSE OF ALLERGIC DISEASES; A METHOD FOR MANAGEMENT IN THE HOME ENVIRONMENT.

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Domestic mites such as *Dermatophagoides* spp. are major producers of allergens, which are responsible for mite allergic diseases, like asthma.
The first step in mite-management is detection of the source of these allergens. In the home environment the detection of a nitrogeous waste-product of the mites -guanine- with the aid of a dip-stick is the method of choice for the laymen. A good correlation exists between guanine and mite-allergens ($r=0.56$), but not between mites and guanine ($r=0.17$). Mite-infested objects should be treated in such a way that either the mites are killed and the old allergens are removed, or the mites as well as allergens are immobilized by the use of mite- and allergen-tight encasings.
The use of such methods in a double-blind placebo-controlled clinical study demonstrated that mite-reduction was near 100% and allergen-reduction was over 50% within 1 year after treatment. Subjective and objective data were showed significant improvements in the active group compared with the placebo-group (symptomscore 47%; $p=0.025$ and total IgE 38%; $p=0.0049$). A further treatment is necessary once or at most twice a year depending on the level of infestation. An expansion of this mite-management system with a method to prevent reinfestation of mites would optimize this already practical concept.

18-075

WATER BALANCE OF THE HOUSE DUST MITE *DERMATOPHAGOIDES PTERONYSSINUS* (ACARI; PYROGLYPHIDAE) IN DIURNALLY FLUCTUATING AIR HUMIDITY

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House dust mites are able to extract water vapour from the air, but only if the relative humidity (RH) is above a certain minimum. This minimum RH-value is called the 'Critical Equilibrium Humidity (CEH)'. We studied the RH and temperature on carpeted floors and in the core of mattresses, where house dust mites live. In these microclimates the RH is commonly below the CEH during most of the day and above it for only a few hours. In a separate experiment we examined how many hours per day the RH must be above the CEH for the mites, to maintain water balance. It was found that a daily period of elevated humidity ($>CEH$) lasting 1 1/2 hours; strongly promotes the survival of a population and that 3 hours above the CEH enables the mites to produce some eggs.

18-076

HOUSE DUST MITES AND STORAGE MITES IN HOMES OF PEOPLE LIVING IN AGRICULTURAL SETTLEMENTS IN ISRAEL

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¹Dept. of Parasitology, Hebrew University-Hadassah Medical School, Jerusalem, ²Israel Meteorological Services, Beit Dagan and ³Clinical Immunology and Allergy Unit, Hadassah Medical Center, Jerusalem, Israel. The correlation between climatic conditions and mite numbers in houses from rural areas was studied. Houses from 13 agricultural communities (kibbutzim and moshavim) in seven geo-climatic areas of Israel, i.e., the coastal region, Samaria-Judean foothills, high mountains, low mountains, Negev desert, northern valleys and central Jordan valley were randomly chosen for examination. A mattress, carpet and sofa were examined in each house. A total of 195 dust samples was collected with a vacuum cleaner during July and August 1995. In addition, two houses from Zova, a kibbutz in the mountains of Jerusalem, and two houses from Palmachim, a kibbutz in the coastal region, were chosen for monthly sampling throughout the year. Mites were present in 97% of the dust samples. The average number of mites per gram of dust in the different localities ranged between 84 and 2,053. Most of the mites were isolated from the carpets and sofas (37.0% and 33.7%, respectively), and less from the beds (29.3%). The maximum number of mites (7,440/g dust) was found from a carpet from Geva Carmel in the coastal region. The most prevalent species of mites were *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, found in 85.6% and 71.3%, of the samples, respectively. The house dust mites (*D. pteronyssinus*, *D. farinae* and *Euroglyphus maynei*) constituted 94.8% of the mites. Storage mites of the sub-order Astigmata (*Blomia*, *Acarus*, *Gohieria*, *Tyrophagus*, and *Rhizoglyphus*), Mesostigmata, Cryptostigmata and Prostigmata constituted 5.2% of the mite fauna. These findings are not significantly different from those of an urban environment. The relationship between mite numbers and climatic conditions, in particular temperature and humidity, will be analysed statistically. The seasonality of the mites in the areas examined, will be discussed.

18-078

RECENT PROGRESS IN BIOLOGICAL CONTROL OF STORED FOOD MITES.

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Biological control which has been developed and proven effective against mites of stored grain and seed is the only biological control method practically used in the stores.

The predator used is *Cheyletus eruditus*, a predatory mite that is commonly found in stores.

Biological control can be used as a preventive measure in empty stores and to prevent or repress pest populations on grain or seed

The predator has lower susceptibility to insecticides than its prey. Differences in susceptibility to insecticides were also found among different strains of *Cheyletus eruditus*. A long-established laboratory strain was most susceptible. A strain, which survived exposure to of PH₃ most resistant. Low susceptibility was also found in strains from the field and from the grain stores. A population of *Cheyletus eruditus* resistant to low temperature was selected. The development of these predators at 12°C is 4 times longer than at 25°C.

18-077

RECENT PROGRESS ON RESEARCH CONCERNING SEX PHEROMONE IN ASTIGMATID MITES

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The female sex pheromones (produced by females and functioning as con-specific male's sexual excitant) have been identified in following four species of genus *Caloglyphus*, including two unidentified; sp. 1, *C. polyphyllae*, *C. rodriguezi* and sp.2, as (2R, 3R)-epoxyneral, β-acaridial, undecane and rosefuran., respectively. These compounds were, however, commonly distributed not only in females, but also in con-specific males and, in extreme cases, also detectable in nymphal stages. In order to understand those phenomena rationally, contents among sexes and developmental stages, and male behavior against both sexes were compared among four species.

Content ratio of female to male varied from 1.4 (sp. 1), 3.4 (*C. polyphyllae*), 6.3 (*C. rodriguezi*) to 8.4 (sp. 2). In nymphal stages of the sp. 1 and *C. polyphyllae*, their pheromone were detectable as a major component, while trace amount in the other two species. Evaluation of male's ability to recognize conspecific females revealed that males of all three species except the sp. 1 could discriminate between females and males.

As a result, the mite sex pheromone was suggested to have evolved from a common compound functioning as male's sexual excitant into the sex linked specific one with biological activity.

18-079

MANAGEMENT OF TERMITE PROBLEMS IN BUILDINGS - CURRENT PRACTICES AND TRENDS IN AUSTRALIA

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The ban on using organochlorines for termite control in Australia since 1 July 1995 has greatly enhanced the development of alternative strategies for the protection of buildings from subterranean termites. A greater diversity of control measures is now available or under development in Australia than in most other countries with termite problems.

Prevention of termite infestations is achieved with:

(1) Physical barriers: Two systems, using stainless steel mesh or graded stones, incorporated into the Australian Standard on termite control; a number of other materials is currently assessed for their suitability in forming effective physical barriers to termite entry.

(2) Chemical barriers: One organophosphate (chlorpyrifos) and one synthetic pyrethroid (bifenthrin) with various registered applications. Other chemicals, such as the novel compound imidachloprid, still under evaluation. Chemicals can either be applied directly to the soil or for slab-on-ground constructions also via a number of reticulation systems. Chemical barriers can also be formed by applying the compounds to other carriers than soil, such as a fibrous blanket or plastic materials.

Control of existing infestations can be achieved by locating and destroying termite nests; dusting termites and their workings with arsenic trioxide (often in conjunction with trap-and-treat systems); and by the establishment or re-establishment of chemical soil barriers. Under evaluation are alternative dusts, a range of bait-systems (matrix + slow-acting, non-repellent toxin) and the use of biological control agents (especially the fungus *Metarhizium anisopliae*). Developments in termite management in Australia focus increasingly on design of buildings and use of resistant materials ("building-out" termites), safer application of chemical barriers, trap-and-treat and bait systems, use of pathogens, and the integration of different control strategies.

18-081

RESPONSE OF DIFFERENT SPECIES OF INSECTS TO MIXTURES OF CARBON DIOXIDE AND/OR NITROGEN IN AIR

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Exposure of different species of insects to 60% CO₂ in air and mixture of 98% nitrogen and 2% oxygen enables a quicker kill to adults (after 2 to 3 days) than does exposure to mixture contained 40% CO₂ in air.

These species are: *Oryzaephilus surinamensis*, which is more tolerant than *Q. mercator*, *Tribolium confusum* is slightly tolerant than *T. castaneum*, *Trogoderma inculsum* is tolerant than *T. grasmani*, *Dinoderus*

porcellus and *D. bitoveolatus* are very sensitive at 60% CO₂ in air and 98% N₂, but at 40% CO₂ in air needs more than 5 days to gain 100% kill of their adults.

These results are due to the relative order of susceptibilities of different insect species to the depletion of oxygen in the mixture.

18-080

METARHIZIUM ANISOPLIAE AS A MYCOTERMITICIDE - LABORATORY BEHAVIOURAL BIOASSAY

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The insect pathogenic Hyphomycete fungus *Metarhizium anisopliae* is a promising biological insecticide for the major pest species of subterranean termites in Australia. The infectious green conidia are mass-produced by solid-state fermentation on rice and the pure dry conidial formulation can be used for control in a number of ways. One promising method is by direct application to kill colonies of mound and tree nesting termites. While inundative application directly to termite nest colonies in mounds or in trees has been shown to be highly effective, indirect control of colonies by treatment of termite feeding sites has been shown to have variable results due to the ability of termites to detect the fungal spores. A laboratory bioassay to measure the relative repellency/attractancy of different baits or bait matrices based on termite tunnelling activity is described. The assay also allows determination of the degree of inhibition of repellency by adding varying amounts of other behaviour-modifying components, or synergists. This assay has been used to study the effects of different strains or isolates of *Metarhizium*, different concentrations and age of conidia, and the addition of various bait matrix components, on tunnelling response of termites. Such data are important to development of baiting systems for termites, which may incorporate pathogens, insect growth regulators, slow toxicants, or combinations of any of these as the main control agent, together with attractants.

18-082

BOOTSTRAP SIGNIFICANCE TESTS FOR A BIOLOGICAL CONTROL EXPERIMENT USING THE MARKOV-RECAPTURE ESTIMATE

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This paper presents the development of a parametric bootstrap significance test used in the analysis of replicated Markov-Recapture results, which were used to monitor an experiment on augmented biological control of *Sitotroga cerealella* in bulk stored corn. Six 10,000 bushel grain bins were inoculated with *S. cerealella*, and three of those inoculated later with the parasitoid *Pteromalus cerealellae*. Markov-Recapture was used to estimate the host populations weekly. The test statistic was the difference between group averages. Population size was first estimated for the *i*th bin (\hat{N}_i), and empirical capture probabilities used to reassign each estimated population \hat{N}_i into capture categories to form each bootstrap replicate. Estimation was performed again using replicates from each bin, and the difference between the group averages noted. The test was considered significant when a difference of 0 fell into the tails of the distribution of group differences. Simulations were performed to examine the power of the significance test. The test was reasonably powerful for the range of data noted in the actual experiment.

18-083

TIME-COURSE MORTALITY AND RADIOSENSITIVITY INDICES IN IRRADIATED *TRIBOLIUM* SPP.

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Effect of gamma irradiation (Cobalt⁶⁰) on the time-course mortality and radiosensitivity indices in adult (1- and 10-days old) *Tribolium* spp. (*T. anophe*, *T. brachycornis*, *T. castaneum*, *T. destructor*, *T. freemani*) was studied. Investigation showed that the longevity was always adversely affected as result of irradiation of 1- and 10-days old adults and was linearly dependent on the dose rate. It also showed that most of adults died within 12 weeks of irradiation at 4- and 5-krad except for 10-days old irradiated adults. In addition, *T. destructor* was markedly more radioresistant than were the other species at all dose levels and had a longer life expectancy. The mean survival times of adults were shorter in female than in the males in most cases for all the species. The radiosensitivity indices did not vary widely among the species and these values decreased as the dose increased in all the species which clearly indicate that the resistance of the species is dose-dependent.

18-084

CROSS-RESISTANCE IN STRAINS OF *RHYZOPERTHA DOMINICA* (F.) (COLEOPTERA: BOSTRYCHIDAE) AND CHANGES IN INSECTICIDE TOLERANCE WITH OR WITHOUT DELTAMETHRIN SELECTION.

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Cross-resistance in deltamethrin resistant strains of *Rhyzopertha dominica* was evaluated with three insecticides used also in stored grain to control pests, permethrin (pyrethroid), pirimiphos methyl and chlorpiriphos methyl (organophosphates). Deltamethrin was used as standard. Ten strains from Brazil (BR1, BR2, BR3, BR4, BR5, BR6, BR7, BR8, BR9 and BR10) were used and one laboratory strain from Imperial College at Silwood Park (UK1). BR4 and UK1 were susceptible and BR6 and BR7 had shown high resistance to deltamethrin. The other strains had intermediate resistance to that insecticide. Changes in tolerance in five of these strains (BR4, BR2, BR6, BR7 and UK1) when exposed or not to selection with deltamethrin at each generation were investigated. The selection like the bioassays was carried out on filter paper, the survivors were cultured and selected again at the next generation. At every third generation adults were bioassayed against deltamethrin. The LD₅₀ of each strain was determined and resistance ratios calculated. The results showed that cross-resistance to permethrin existed in strains BR6 and BR7 strains, but no clear evidence of cross-resistance to pirimiphos methyl or chlorpiriphos methyl was found in any of the strains tested. In the selection experiments, resistant strains reared without selection decreased in their susceptibility while susceptible ones were not changed. Also selected strains changed in their tolerance to this insecticide.

18-085

INSECTICIDE RESISTANCE IN *RHYZOPERTHA DOMINICA* (F.) (COLEOPTERA: BOSTRYCHIDAE) INFESTING STORED GRAINS IN BRAZIL.

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The failure to control *Rhyzopertha dominica* (F.) in Brazil was recorded by the first author in many storage units where the insecticide deltamethrin was used repeatedly to protect grains. Samples from these and other pest-populations were taken to investigate their tolerance. Ten strains from Brazil (BR1, BR2, BR3, BR4, BR5, BR6, BR7, BR8, BR9 and BR10) and one laboratory strain from Imperial College at Silwood Park (UK1), were bioassayed on filter paper against deltamethrin (K-otec 25 EC). Another series of bioassays were carried out on some of the strains (BR4, BR2, BR6 and BR7 and UK1) where adults were bioassayed in each one of four age ranges (1-10, 11-20, 21-30 and 31-40 days after emergence). Eggs collected from untreated individuals in each group were reared through to adulthood and these assayed also 10 days after emergence. The LD₅₀s were determined and resistance ratios calculated. The results showed two strains, BR4 and UK1, were the most susceptible and these were taken to represent the normal tolerance level of this species to deltamethrin. Two strains, BR6 and BR7, showed high resistance factors of 105 and 700 respectively. The other strains, BR1, BR10, BR5, BR8, BR2, BR9 and BR3 exhibited a range of resistance factors up to 74 times greater than the most susceptible strain. There were no statistical differences among the age groups in all the strains tested and also no significant differences between the progeny from each age group. These results mean that comparable data may be achieved with populations of differing age structure and that the susceptibility of the progeny was also independent of parental age.

18-086

POTENTIAL BIOLOGICAL CONTROL OF BRUCHIDS INFESTING STORED DRIED LEGUMES WITH THE GENERALIST PREDATOR *XYLOCORIS FLAVIPES* (REUTER) (HEMIPTERA: ANTHOCORIDAE)S. Sing^{1,2}, R.T. Arbogast¹, J.H. Brower³, R.K. Stewart²

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F₁ emergence of the pest Bruchidae *Callosobruchus maculatus* (Fabricius), *Callosobruchus chinensis* (Linnaeus), *Callosobruchus analis* (Fabricius), *Zabrotes subfasciatus* (Boheman), and *Acanthoscelides obtectus* (Say) infesting the common bean *Phaseolus vulgaris* (L.), chickpea *Cicer arietinum* (L.), and cowpea *Vigna unguiculata* (L.) Walp. was significantly reduced by the presence of *Xylocoris flavipes* (Reuter) in test arenas. Both predator density and predator introduction time impacted the number of emerging F₁ bruchids.

Suppression of pest bruchids was enhanced by the combined treatment of *Xylocoris flavipes* and various strains and species of the pesticide-resistant and pesticide-susceptible hymenopteran larval parasitoids *Anisopteromalus calandrae* (Howard), *Pteromalus cerealellae* (Ashmead), and *Choetospila elegans* Westwood. Biocontrol efficacy of a pesticide-susceptible laboratory strain of *Xylocoris flavipes* was not significantly different from that of the pesticide-resistant field strain of the predator.

18-087

STUDY ON A NEW METHOD CONTROL RETICULITERMES DAMAGES BUILDING

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The method of drilling and putting baits is a new method in control *Reticulitermes* damaging building. The result of control showed 96.58% on yellow chest termite and 100% on black chest termite. The operation process is simple. It can be used in the four seasons of the year. It damages buildings slightly and doesn't make environmental pollution

Key Words:

Reticulitermes, the method of drilling and putting baits

18-088

ANTI-INVADING METHODS AGAINST GREGARIOUSLY OVER-WINTERING STINK BUGS IN JAPANESE HOUSES

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A great number of stink bugs, mainly *Halyomorpha halys* and *Menida scotti*, gathered to invade the buildings at the many mountainous areas of Japan in every year. For their bad smell giving out they are disliked as nuisance at the hotels, schools, offices and dwelling houses. From 1988 we have been studying the biology of coming-fly and control of these house-invading stink bugs at some mountainous areas in Toyama Prefecture, central part of Japan.

House-invading activities of *H. halys* began in late September and ended in late November. The peak flight activity of *H. halys* was observed on mid-October at the altitude of 300 m. *M. scotti* gathered around the buildings later than *H. halys* and the peak activity was observed on early November at the altitude of 600 m. The "slit-trap" was proved to be effective as an attractant for these bugs. The application of concentrated cyphenothrin or repellent (deet) to window frames was effective to prevent the invasion of these bugs. Covering by cyphenothrin-treated nets over the wooden houses was also effective.

18-089

THE PESTICIDAL EFFECT OF SOME SELECTED BOTANICALS ON REDUCING INFESTATIONS BY CALLOSOPRUCHUS SPECIES

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Callosobruchus maculatus (bruchid beetle) is a serious insect pest of grain legumes in Sri Lanka along with *Callosobruchus chinensis*. In an investigation to explore the possibility of using botanicals, it was found that *Cymbopogon citratus* (L.), *Cinnamomum camphora* (L.) and *Monodora myristica* (L) significantly lowered egg hatchability of two *Callosobruchus* species. No Plant powder was effective in controlling the adults. The current status of affairs of using botanicals to manage *Callosobruchus* is reviewed.

18-090

BIOACTIVITY OF POWDERS FROM SOME PLANTS ON *ZABROTES SUBFASCIATUS* (BOH.) (COLEOPTERA: BRUCHIDAE)

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The repellency, oviposition and survival of the Mexican bean weevil, *Zabrotes subfasciatus* (Boh.) were evaluated under laboratory conditions with bean grains treated with powders obtained from fruits of neem (*Azadirachta indica*) and pepper (*Capsicum frutescens*); from leaves of eucalyptus (*Eucalyptus citriodora*), chinaberry (*Melia azedarach*) and castor bean (*Ricinus communis*); and from a mixture of leaves, flowers and fruits of the Mexican tea (*Chenopodium ambrosioides*). Each powder was mixed with bean grains and kept in a small plastic box (6 cm diameter). The repellency of the powders was determined in a double choice experiment between treated (0.3 g of powder per 10 g of bean) and untreated boxes (only beans) kept in an arena. The number of individuals in each treatment was evaluated 24 h of insect release. The oviposition and survival were evaluated by infesting 5 couples of *Z. subfasciatus* (1-day old) per recipient (0.6 g of powder per 20 g of bean) for 5 days. Boxes containing only beans were used as controls. The number of live insects and eggs per recipient were counted daily. The most repellent plant species were *E. citriodora* and *C. ambrosioides*, followed by *M. azedarach*. The only treatment that showed significant effect on survival and oviposition of *Z. subfasciatus* was *C. ambrosioides* which caused 100% mortality one day after infestation. The insecticide activity threshold was determined for this plant species; data showed that the bruchid mortality was higher than 90% even at the concentration of 0.075 g of powder per 20 g of bean.

18-091

BUILDING MEASURES AGAINST COCKROACHES

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Cockroaches are common pests in buildings around the world. Because of restricted use of insecticides and resistance of cockroaches, building (preventive) measures are becoming more important. A study has been done on building measures to reduce transport and reproduction of cockroaches in bakeries in the Netherlands. The study was limited to the cockroach species *Blattella germanica*, *Blatta orientalis* and *Periplaneta americana*. This study involved a literature investigation and a case study. Dispersal of cockroaches can take place through cracks of 0.5*1 mm². Reproduction is dependent on temperature and relative humidity of the air. Cracks and presence of niches can be reduced by building technology. The reproduction of cockroaches is influenced by physical measures. A statistical model was constructed to relate reproduction from *B.orientalis* to physical variables ($\alpha = 0.05$) and that may be used to evaluate a physical measure. Three bakeries in the Netherlands were investigated. All were infested. The hypothesis based on the investigation reads that infestations take place in the stocks of goods via incoming goods and means of transport. From there, transport take place within the bakery. Building measures based on this hypothesis are:

1. Stocks of goods must be cooled and dried. Transport of cockroaches can be reduced by closing cracks and crevices.
2. In bakeries the presence of niches must be prevented first. Remaining niches must be made accesible for pest control or can be made unsuitable for reproduction by building physical measures. But first cause and development of infestations need to be studied.

18-093

A NEW MICROSPORIDIAN PATHOGEN OF *TROGODERMA VARIABLE* (COLEOPTERA: DERMESTIDAE)

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Protozoan diseases are commonly found in stored product beetle pests. *Trogoderma* spp. are known to harbour protozoans from the genera *Mattesia*, *Adelina* and *Pyxinia*. A single incidence of an unidentified microsporidan was reported from *T. simplex* in California. No microsporidan pathogen has previously reported from *T. variable*.

Laboratory cultures of *T. variable* that were initiated by insects collected in New South Wales and Western Australia were found to be infected with a microsporidan pathogen. Here we report on a study of its taxonomic status and potential value in biological control.

Features of the life-cycle and the ultrastructure place the organism in the genus *Nosema*. On the basis of host and tissue specificity and features of the spores such as the size, polar filament length and arrangement as seen in thin sections, it was concluded that the present species has not been described previously.

This *Nosema* pathogen is infectious per os, attacks the larval fat body and can substantially reduce the rate of increase of the population. It causes little direct mortality even at doses as high as 10⁷ per g of medium. Sublethally infected adults are readily produced and the infection reduces their fecundity. No evidence of transmission in or on the egg was found.

The fact that the disease can substantially reduce the rate of population increase in *T. variable* suggests a possible role for *Nosema* in biological control. The disease is too slow acting and not sufficiently lethal to be a good microbial insecticide. However, introducing the disease into the general population may be effective in reducing the abundance of the pest within the general environment.

18-092

EFFECTIVENESS OF FOUR ESSENTIAL OILS AGAINST *CORCYRA CEPHALONICA* (St.) (LEPIDOPTERA : PYRALIDAE)

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Different concentration of four essential oils viz., geranium oil (*Palogonium graveolens*), palmarosa oil (*Cymbopogon martini*), citronella oil (*C. winterianus*) and lemongrass oil (*C. flexuosus*) were tested against rice moth, *Corcyra cephalonia* Stainton. The toxicity of essential oils against eggs was as lemongrass citronella palmarosa geranium. The calculated LC₅₀ values of different oils for eggs ranged from 0.4741 to 1.9529 per cent. Significant reduction in adult emergence was recorded when newly hatched larvae were developed on treated food with oils. The essential oils had feeding deterrent activity. The weight of food ingested and digested and the weight gained by the test insects were reduced when fed on treated food. The approximately digestibility (AD), efficiency of conversion of ingested food to body substances (ECI) and efficiency of conversion of digested food to body substances (ECD) were also decreased.

18-094

INFESTATION EFFECT OF *Tenebrio molitor* (COLEOPTERA: TENEBRIONIDAE) ON AMINOACIDS CONTENT OF MEAT AND BONE MEAL

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Tenebrio molitor infestation alters the chemical composition and nutritional value of stored products. The present work was aimed to verify the essential aminoacid levels of meat and bone meal submitted to several *T. molitor* infestation levels. Five infestation levels were tested (0, 10, 20, 30 and 40 larvae/ 100g of meat and bone meal), with four replicates each treatment. Two samples of each treatment had aminoacids compositions analysed at the 1st, 30th and 60th day after infestation. The first sample consisted of grounded larvae and meat and bone meal whereas the second sample had the larvae removed from it. The aminoacids studied was methionine, cysteine, lysine, tryptophan, arginine, histidine, phenylalanine, leucine, isoleucine, valine, and threonine. The results showed that the infestation of *T. molitor* changed the aminoacids meat and bone meal content, mainly on the samples with larvae present.

18-095

ACTION OF *Tenebrio molitor* (COLEOPTERA: TENEBRIONIDAE) ON THE MEAT AND BONE MEAL MINERAL CONTENT.

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The present work was aimed to verify the changes on the mineral content of meat and bone meal submitted to several infestation levels of *Tenebrio molitor* larvae. The experiment was carried out with 0, 10, 20, 30 and 40 larvae/ 100g of meat and bone meal with four replicates for each treatment. Calcium, phosphorus and sodium levels were analysed of samples of each treatment at the 1st, 30th and 60th day after infestation. The first sample consisted of grounded larvae and meat and bone meal whereas the second one had the larvae removed from the meat and bone meal. The results showed that the infestation of *T. molitor* changed the meat and bone meal chemical composition, mainly on the mineral value of the highest infestation level.

18-097

THE VARIETAL CHARACTERISTICS OF WHEAT INFLUENCING POST-HARVEST INFESTATION BY *SITOPHILUS GRANARIUS* (COLEOPTERA: CURCULIONIDAE) AND CAUSED LOSSES

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Twenty-eight wheat varieties registered in the Czech Republic were tested under laboratory conditions for their susceptibility to post-harvest infestation by *Sitophilus granarius*. The criteria used were: the number of the F₁ progeny, the duration of development, the dynamics of adult emergence and the weight losses of grain caused by infestation. The factors affecting cultivar susceptibility were studied.

Varieties of best technological qualities were least susceptible to *S. granarius*. The higher tolerance of these cultivars to infestation appears to be connected with their physical and chemical characteristics. Negative correlations between pest progeny production and protein content, vitreousness and hardness of kernels were found. In all varieties, the results fluctuated depending on the year of the harvest.

18-096

INFESTATION EFFECT OF *Tenebrio molitor* (COLEOPTERA: TENEBRIONIDAE) ON NUTRITIONAL VALUE OF MEAT AND BONE MEAL

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The beetle *Tenebrio molitor* is an important pest on stored animal feed such as poultry and swine. The present work was aimed to verify the energy and protein contents of meat and bone meal submitted to several beetle infestation levels. Five infestation were tested (0, 10, 20, 30 and 40 larvae/ 100g of meat and bone meal), with four replicates for each treatment. Energy and protein crude compositions of sample of each treatment were analysed at the 1st, 30th and 60th day after infestation. A sample consisted of grounded larvae and meat and bone meal whereas other one had the larvae removed from it. The results showed that the infestation of *T. molitor* changed the chemical composition and the nutritional value, of the feed. Therefore, the results suggest that infestation of *T. molitor* could be used to increase the protein value of the stored animal feed.

18-098

TRAPPING RESPONSE OF THREE SPECIES OF *TRIBOLIUM* TO VARIOUS WINDOW TRAPS

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The trapping experiments were conducted on *Tribolium castaneum*, *T. confusum* and *T. destructor*, which are the most important tenebrionid pests in stores and households in the Czech Republic. Three commercial types of window traps (Agrisense), i.e. box-shaped sticky traps lured with aggregation pheromone, were tested. Two of these had narrow entry channels.

The traps were more efficient for *T. confusum* than for *T. castaneum*, whereas they were completely ineffective for *T. destructor*. Their efficiency was strongly affected by the competing factors of surrounding environment, alternative food being most important. Two types of trapping rate were observed which were triggered by the presence (linear response) or absence (exponential response) of food in the arena.

18-099

INSECTICIDE RESISTANCE IN *TRIBOLIUM CASTANEUM* AND *TRIBOLIUM CONFUSUM* (COLEOPTERA: TENEBRIONIDAE) IN THE CZECH REPUBLIC

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Field strains of *Tribolium castaneum* (8 samples) and *T. confusum* (3 samples), collected from various storages, bakeries and flour mills in the Czech Republic, were surveyed for resistance to malathion, pirimiphos-methyl, fenitrothion and chlorpyrifos-methyl.

Dose-response test procedure was used (FAO method No. 15, slightly modified). Adult beetles (mostly F₃ generation, 20-30 days old) were confined to an insecticide-impregnated filter paper placed in a Petri dish for 24 hours.

Low-level resistance to four organophosphates was found in almost all field strains of *T. castaneum* (resistance factor >2). The highest resistance observed was the response to pirimiphos-methyl (resistance factor 5 to 23), which is well corresponding with a frequent and long-term usage of pirimiphos-methyl in the Czech Republic. The highest susceptibility was found to chlorpyrifos-methyl: insecticides containing chlorpyrifos-methyl are only used within a few recent years in the Czech Republic.

Low-level resistance to pirimiphos-methyl and to malathion (slightly lower than to pirimiphos-methyl) was found in all three field strains of *T. confusum*, while resistance to fenitrothion and chlorpyrifos-methyl in field strains of *T. confusum* was not significantly higher than in the susceptible laboratory strain.

The results of this study have shown that low-level resistance to various organophosphates, especially to pirimiphos-methyl, is present and widespread in the populations of *T. castaneum* in the Czech Republic.

18-101

DIFFERENCE OF MITE NUMBER AND ALLERGEN IN SUMMER AND WINTER BETWEEN WITH AND WITHOUT PETS (CAT AND DOG) IN A HOUSE

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Mite numbers and allergens (Der1: allergens derived from feces of *Dermatophagoides pteronyssinus* (Derp1) + *D. farinae* (Derf1)) were compared in houses in Tokyo in summer and winter in which non-pet (A), single pet (cat or dog: B) and plural pets (cats or dogs or both: C) were kept.

In winter, 1994, 30 houses (10A, 10B, 10C) were selected for collecting house dust using a vacuum cleaner. The dust was sieved through 16 and 200 mesh and 0.5g fine dust on 200 mesh was examined by a centrifuging method using saturated NaCl for mite research, while dust was sieved through 48 mesh and 0.1g of fine dust was analyzed for Der1 amounts by an enzyme-linked immunosorbent assay.

There were no significant differences in mite numbers and Der1 amounts between those in houses A and B-C with average mite numbers/0.5g-dust of 980.2 (A), 845.8 (B) and 424.2 (C), and average Der1 amounts of 21.4 µg/g-dust (A), 27.2 (B) and 16.7 (C). Pyroglyphidae occupied 76.3-94.6% of the total mites in houses A-C, although Derp1 had 50.9-84.4% of Der1.

In summer, 1995, 39 houses (14A, 17B, 8C) were examined for mite numbers and Der1 amounts in the same way as in winter. There were no significant differences between those in A and B-C. The average mite number/0.5g-dust was 881.4 (A), 961.1 (B) and 408.9 (C), while average Der1 amount 27.1 µg/g-dust (A), 23.5 (B) and 23.3 (C). Pyroglyphidae and Derp1 accounted for 83.4-93.4% and 67.2-89.7%, respectively.

18-100

TOXICITY AND PROTECTANT POTENTIAL OF MAJOR COMPONENTS OF ESSENTIAL OIL OF THREE *OCIMUM* SPECIES AGAINST STORED PRODUCT COLEOPTERAD. Obeng-Ofori, Ch. Reichmuth, A.J. Bekele¹ and A. Hassanali¹

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The use of locally-available plant materials to limit insect damage in stored foodstuffs is common practice in traditional farm storage in developing countries. *Ocimum* plants grow widely in India and parts of East Africa and have traditionally been used as insect repellents against mosquitoes. Local farmers also mix stored foodstuffs with dry leaves of these plants for protection against insect pest damage in storage. Essential oil extract was isolated by steam distillation from leaves, inflorescences and succulent stems of *Ocimum kenyense*, *O. suave* and *O. kilimandscharicum* (Labiatae) and analysed by GC-MS techniques and spectral comparison with synthetic standards. 1,8 cineole, eugenol and camphor were identified as the major components comprising 40, 60 and 70% of the total collection, respectively.

The bioactivity of each compound against *Sitophilus granarius*, *S. zeamais* and *P. truncatus* was evaluated in the laboratory by contact toxicity, grain treatment and repellency assays. Each compound applied topically or impregnated on whole wheat and maize grains was highly toxic to the three species. Development of eggs and immature stages of the beetles within grain kernels as well as progeny emergence was completely inhibited in treated grain. Eugenol and camphor were also highly repellent to the beetles with overall repellency in the range of 80-100%. There was, however, highly significant loss of toxicity after only 24 h following treatment.

18-102

VEGETABLE OILS FOR PROTECTION OF PIGEONPEA, *CAJANUS CAJAN* (L.) MILLSP. AGAINST *CALLOSBRUCHUS CHINENSIS* (L.) IN STORAGE ECOSYSTEMS

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Effectiveness of vegetable oils coconut, groundnut, mustard and soybean used at two doses of 5 and 7.5 ml/kg seed as surface protectants of pigeonpea, *Cajanus cajan* (L.) Millsp. against *Callosobruchus chinensis* (L.) was investigated for two years. During both the years pigeonpea seed treated with both doses of all the tested oils resulted in reduced mean per cent seed damage and weight loss at all the testing levels of 2, 4, 6 and 8 months as compared to control. The level of mean per cent damage in the pooled data in coconut, groundnut, mustard and soybean oils was upto the tune of 0.0 to 0.0, 0.0 to 0.0, 0.0 to 0.0 to 2.15 and 0.0 to 8.83% as compared to 30.34, 67.23, 97.83 and 94.40% in control after 2, 4, 6 and 8 months of storage, respectively. After 2 and 4 months of storage interval, practically no weight loss was observed in the treated seeds at both the oil doses. After 6 months of storage in the pooled data, higher doses of coconut (0.0% weight loss), groundnut (0.0%), mustard (0.0%), soybean (0.0%) and lower dose of coconut (0.18%) and groundnut (0.0%) proved superior and equieffective and in reducing seed weight less than lower dose of mustard (0.97%) and soybean (1.82%). After 8 months, seed treatment with both higher and lower doses of all oils (0.0 to 0.91%) except lower dose of soybean (7.44%) were at par and proved superior in reducing mean per cent weight loss.

18-103

EFFECTIVENESS OF BENZYL ALCOHOL IN THE CONTROL OF HOUSE DUST AND STORED CROPS MITES (ACARI ASTIGMATA)

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Benzyl Alcohol (BA) is recognized as pharmaceutic aid on account of its bacteriostatic properties and considered non-toxic for man at low concentration (oral LD₅₀ : 500mg/Kg). Its acaricide effects were tested on the house dust and storage mites *Dermatophagoides pteronyssinus* (Trouessart), *D. farinae* Hughes, *Euroglyphus maynei* (Cooreman), *Tyrophagus putrescentiae* (Schränk), which are pests of stored crops and are responsible for atopic allergy.

For each species from stock cultures in optimal growth conditions 150 eggs and 1100-1500 motile stages (7-9 replications of about 150 specimens) were uniformly nebulized (80 µl on about 100 cm²) with a 3.25 % water solution of BA. The effect on motile stages was evaluated at room temperature (15°-19°C) in the first 24 hours after the treatment. The eggs were kept in a climatic cabinet at about 22°C for a period longer than that necessary for their hatching and observed once a day. The humidity was always high (> 80%) because the tests were carried out on units surrounded by wetted cottonwool to prevent the mites escaping.

Immediately after spraying almost all the mites appeared immobilized. The highest mortality on motile stages was recorded after 24 hours and was about 83% for *E. maynei* and *D. farinae*, 84% for *D. pteronyssinus* and 87% for *T. putrescentiae*. The effectiveness of BA was lower on the eggs with a 56% of hatchability for *T. putrescentiae* and 25-30% for the other species. However, the natural mortality under the tested conditions of *T. putrescentiae* was 1%, while for the others 8-9%. BA also appeared to exert a repellent effect and the treated surface were less colonized by mites. Our similar previous experiment on *D. farinae* at 20°-25°C recorded a 96% mortality.

These results suggest a promising use of BA to control mite populations in the home and in storage environments.

18-105

EVALUATION OF A METHOD OF FILTH-TEST USED FOR DETECTION OF SOLID MATTER IN MEAL

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This method involves "chemical digestion" of meal by nitric acetate solution, flotation of solid matter with ethyl alcohol and benzene, trapping in a Wildman flask and vacuum filtration.

Results showed a mean detection rate of 96% of insect fragments counted and introduced in 15 programmed repetitions. Possible causes of error at different stages of the procedure are discussed.

The same method was also evaluated on chestnut meal, giving good results.

18-104

TERMITES AND WOODEN STRUCTURES IN MONREALE CATHEDRAL

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The wooden structures in the roofs at Monreale Cathedral have suffered serious attacks by the termite species *Reticulitermes lucifugus* (Rossi) (Isoptera, Rhinotermitidae).

The first hotbeds of termites were found in 1978 under the mosaic surface resting on mortar made up of chalk "reinforced" with barley straw.

Subsequently, after oriented sampling, infestations were found on almost all the structures, especially the north-facing ones, mainly in the ends of the trusses in the nave and in the beams in the aisles and transept. The damage was extensive.

Termite nests were found near the baptistery, on one side, and in the vicinity of the north portico and the Angels' Arch on the other.

Two methods were used for the safeguarding of the monument: one tending to drive termites away from the infestation area and to create environmental conditions unfavourable to their survival, the other aiming to destroy the termite populations present. The work to achieve these aims was decided in cooperation with the planners of restoration work and carried out in cooperation with the director of works.

Among the main operations for disinfestation and tutelage we can mention:

1. Separation of the main structures of the roof from those of the ceiling.
2. Creation of an air gap between the two structures.
3. Aeration of ends of the trusses and beams by means of appropriate niches.
4. Aeration between the tiles and the roof planks.
5. Curative and preventive treatment of all wooden material with appropriate insecticides.
6. Creation of a trench under the north portico, to interrupt movement of termites from the nests to the "grazing" areas and to reduce rising damp in the building and isolate the town drain.

18-106

DISTRIBUTION AND DENSITY OF HOUSE DUST-MITES *DERMATOPHAGOIDES* SPP. (ACARINA: PYROGLYPHIDAE) IN THE MATTRESSES OF TWO DISTRICTS IN ROME, ITALY

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House-dust mite infestation was studied for the first time in Rome by Bigliocchi & Maroli (*Aerobiologia* 11: 35-40, 1995): 87.8% of the sampled houses were positive for dust-mites. The mattress was significantly the most infested (71.1%) of the tested sites. *Dermatophagoides farinae* was the most abundant species (53.1%), followed by *Glycyphagus domesticus* (34.5%), *D. pteronyssinus* (5.2%), and *Euroglyphus maynei* (0.2%).

The present study deals with the distribution and density of *D. farinae* and *D. pteronyssinus* in the mattresses of two districts in the city of Rome. Weekly samples of dust were collected during the period of high mite density. A total of 4,179 live and dead mites was collected. In the two examined areas, a difference in species distribution was observed. *D. farinae* was always the prevalent species, it was 94.7% in area A and 64.1% in area B; only in the two out of 10 houses was *D. pteronyssinus* the most abundant species (71.4% and 45.8%). Housekeeping practices were investigated by interviewing the tenants; correlation analysis revealed a significant relationship between these practices and mite density. The poorest housekeeping practices produced the heaviest mite infestations, while the good ones were associated with low densities.

Key words: *Dermatophagoides pteronyssinus*, *D. farinae*, house-dust mites, distribution, relative humidity, housekeeping, Rome.

18-107**GENERAL REMARKS ABOUT STORED PRODUCT PROTECTION AS AN INTEGRATED SYSTEM**

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In recent years pest control as a whole and stored product protection specifically is reconsidered under new priorities. Whereas efficacy held the most important position in the decision process as to which method of control or prevention should be applied, other aspects like workers' safety, chemical residues in treated products, pollution, and total energy consumption gain more and more importance. Moreover, due to massive memory capacities of computers and cheap accessibility for people, plenty of various information concerning the behaviour and development of pests, depending on type of product, the dosage of chemicals for control and their residue building can be kept ready in huge data banks around the world connected via internet or world wide web. Expert systems can be expected to cover plenty of cases and situations of pest infestations and their control. These new developments require a detailed concept of how to proceed in a specific situation and how to standardise the methods. The problem analysis has to include a broad investigation and a thorough search for the best combined solution amongst physical, biological, chemical, and technological procedures.

18-109**INERT DUSTS - REVIVAL OF AN AGENT AGAINST PEST INSECTS IN STORAGE**

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Fine inert dusts have the property to kill insects within a rather short time. This knowledge dates back into history being applied in ancient cultures when mixing ashes and sand with stored agricultural products against pest insects. The application of silica was presumably interrupted when worker safety regulations forced a limited use to prevent health risks. Lately, new products were introduced with amorphous and calcinated diatomaceous earth. These compounds could help to partially replace residue building chemical insecticides. In Australia these dusts are effectively used since some years. Experiments in the laboratory under Central European climate and with grain revealed that the efficacy of the earths is strongly linked to the relative humidity in the store. Difficulties with complete control exist with the granary weevil *Sitophilus granarius*. Other pest insects like the warehouse moth *Ephesia elutella*, the saw-toothed grain beetle *Oryzaephilus surinamensis* and the confused flour beetle *Tribolium confusum* die a few days after exposure. Possibilities to use these dusts for empty room treatment and for sanitation of crevices with hiding pest insects are discussed.

18-108**A SIMPLE AND CHEAP ACOUSTIC DEVICE TO DETECT IMMATURE STAGES OF THE INTERNAL FEEDERS *SITOPHILUS GRANARIUS* AND *SITOTROGA CEREALELLA* IN GRAIN**

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National and international grain trade suffers from the fact, that produce infested with internal feeding stages of insects like the granary weevil *Sitophilus granarius* and the Angoumois grain moth *Sitotroga cerealella* are delivered into the market as insect free. The receiver of the goods faces expensive control measures like fumigation of the whole bulk on emergence of these insect pests some weeks after loading.

Sieving of aliquot samples prior to loading is the standard method for inspection leaving behind the described uncertainty.

An apparatus is presented which is capable to detect feeding larvae inside infested grain kernels by their frass activity by use of a very sensitive microphone. The sensitivity is sufficient for one larva in 10 kg of grain or in other words one larva in a distance of about 20 cm. The low cost instrument should be used without too much background noise from the environment for instance in a bureau of a silo or flour mill.

18-110**BIOLOGY AND BEHAVIOUR OF THE TOBACCO BEETLE *LASIODERMA SERRICORNE* (FABRICIUS) (COLEOPTERA: ANOBIIDAE)**

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The tobacco or cigarette beetle *Lasioderma serricorne* creates world wide great losses in stored tobacco, drugs and spices. On the leaves, the feeding activity of the larvae results in holes of 2 mm diameter, which spoil cigars of high value.

Tobacco, tobacco paper, bran and a mixture of bran and tobacco were tested as suitable breeding substrates for the beetle. Abiotic factors like temperature and humidity were varied. The developmental time was registered depending on sex. It was shown that the females preferably deposited their eggs in the area of the central vein of the tobacco leave. At this site the larvae also preferred feeding. The length of the males (2.4 ± 0.2 mm) differed significantly from the corresponding size of the females (3.0 ± 0.3 mm).

18-111

AN EASY APPLICABLE METHOD TO EVALUATE NATURAL AND SYNTHETIC REPELLENTS AGAINST STORED PRODUCT PEST COLEOPTERA AND LEPIDOPTERA.

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The use of plant products as repellents, deterrents or natural insecticides is one alternative method in stored product protection which is currently under investigation. Frequently, natural compounds which are promising in laboratory studies show low efficacy under practical conditions. To overcome this problem, a method was evaluated to study the long term effect of natural compounds on oviposition site selection and feeding behaviour of both lepidoptera and coleoptera infesting stored products. A box containing two glass vessels with feeding substrate were placed inside an experimental room under controlled temperature and humidity conditions (fumigation chamber, glass cube or wire cage). The vessels were arranged upside down with the rim on the top of spacers to enabeling the test insects to enter underneath, feed and oviposite on the test substrate. In one vessel a dispenser saturated with the test compound hung above the substrate. In this two-choice situation, the insects were released outside the vessels. After several days the number of insects on the feeding substrate were counted. Moreover, the substrate was incubated to determine the development of progeny produced by the insects. In experiments conducted with pyralid lepidoptera, dermestid coleoptera and various essential oils, always a lower number of insects was found on the feeding substrate compared with the controls, but the number of progeny produced was not significantly different. The correlation of substrate selection, oviposition site selection and the number of progeny deposited are discussed.

18-113

RECENT COLLECTED MITE PESTS OF FOOD PRODUCTS (ACARI: ACARIFORMES AND PARASITIFORMES)

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Twenty-six families representing two orders and four suborders of mites infesting stored food products are reported. The identified species, country origin, date collected, and infested foods are presented in a table.

Some of these mites collected or closely related species have great medical significance and are important to public health, causing contact dermatitis and other allergic reactions. Ways and means in which these mites are transmitted causing infestations are discussed.

18-112

DISTRIBUTION OF DELTAMETHRIN FORMULATIONS IN VARIOUS SOILS WHEN APPLIED FOR SUBTERRANEAN TERMITE CONTROL
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Deltamethrin emulsifiable concentrate (EC) and soluble concentrate (SC) diluted in water at 0.1% active ingredient (AI) were applied to loamy sand and silty clay soils at 1.52 liter per application point under 206.89 kPa pressure. Each formulation had 4 treatments and each treatment was replicated three times. An experimental unit for each treatment was a plywood box (1.22 m X 1.22 m X 0.61 m) filled with loamy sand or silty clay loam soil and covered with a 7.6 cm concrete slab. A 1.3 cm hole at the center of each concrete slab was made to apply deltamethrin. The insecticide was applied using a sub-slab injector equipped with a 10.2 cm single bore straight tip. Four soil cores measuring 0.61 m in length and 2.54 cm in diameter were removed from each unit with cores 1, 2, 3 and 4 located 2.5, 15.2, 30.4 and 45.6 cm below the bottom of the concrete slab, respectively. Each core was divided into 4 soil samples (15.2 cm interval). Deltamethrin from soil samples was extracted using methanol and analyzed by high performance liquid chromatography (HPLC). Data were analyzed by Proc GLM:Repeated Measures Analysis, ANOVA. The results indicated that neither the formulations nor the combinations of soils and formulations yielded significant differences in vertical or lateral distribution of deltamethrin. In terms of vertical penetration, maximum amounts of deltamethrin (70.7-169.3 µg/g of soil) were detected directly below the injection point regardless of soil type or formulation. Relative to lateral distribution, the highest amounts of deltamethrin (2.7-72.2 µg/g of soil) were found only at 2.5 cm depth combined with a horizontal distance of 15.2-30.4 cm from the injection point.

18-114

ARBOREAL AND LEAF LITTER SPECIES OF N. AMERICAN HARVESTMEN AS NOCTURNAL PREDATORS IN ROADSIDE AND URBANIZED ENVIRONMENTS (ARACHNIDA: OPILIONES).

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During a research study of nocturnal field predation by Opiliones on various arthropods, several species were found to be quite numerous in previously unreported and novel habitats. One unusual type of habitat was the bases of lighted interstate highway signs; 3 harvestmen species were collected in significant numbers in this "habitat" during regular nocturnal collections in Northeastern Ohio from July through September 1990, and in other collections in following years. The harvestmen were apparently drawn to these areas by the many insects attracted to the lighted signs, which were 50 meters or more from typical mesic hardwood forest habitats from which the most frequently collected species, Leiobunum vittatum, has been previously reported. A laboratory maintenance technique for L. vittatum and another opilionid species, Hadrobunus maculosus, will also be described.

18-115

SPERM NUMBERS AND REPRODUCTIVE BEHAVIOUR IN MUTANT AND IRRADIATED MOTHS *EPHESTIA KUEHNIELLA* (LEPIDOPTERA: PYRALIDAE)J. Koudelová, P.A. Cook¹

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Quality of sperm transfer plays an important role in efficiency of genetic methods for suppression of Lepidoptera populations. We have examined competitiveness of mutant and/or irradiated males of *Ephestia kuehniella* by counting sperm transferred to female via spermatophore. BL-2 males, trans-heterozygous for two sex-linked recessive lethal mutations, *sl-2* and *sl-15*, produced 50% fewer eupyrene (fertile) and apyrene (non-fertile) sperm per spermatophore in comparison with wild-type males. However, the ratio of apyrene to eupyrene sperm was the same in both groups (9,5:1).

In the second experiment, late pupae heterozygous for one of the mutation, either *sl-2* or *sl-15*, were treated with different doses of gamma rays: 150, 175, 200, 250 and 350 Gy. Irradiation significantly decreased percentage of the pupal emergence at doses higher 175 Gy. Mating success of males was reduced at all doses excepting 200 Gy by about 50%. After irradiation with the dose of 175 Gy, the mean number of both eupyrene and apyrene sperm increased, even exceeding those in untreated wild-type males. At higher doses used, the numbers of both sperm decreased significantly to the maximal reduction of about 25% at 350 Gy.

The mean time required for copulation increased with the increasing dose. There was a tendency in the relationship between the number of sperm transferred and copula duration: the lower the sperm number the longer the copulation.

18-117

THE ANALYSIS OF REFUGE POPULATIONS OF *ORYZAEPHILUS SURINAMENSIS* IN MODEL GRAIN BINSR.J. Brown, C.A. Malcolm, R.A. Nichols and P. L. Mason*¹

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Oryzaephilus surinamensis is an important stored product pest. DNA based markers have been identified and used in conjunction with model grain bins to facilitate analysis of the structure and dynamics of heterozygous insecticide resistant populations within grain stores, in the presence of insecticide. The model grain bins were designed to mimic features of real grain stores, notably, the varying levels and surface areas of insecticide treatments and the presence of refuges. This work has shown that the presence of untreated refugia allow *O. surinamensis* individuals to survive treatment and reproduce to maintain long term residual populations. The data from the genetic markers showed that some insect refuges contained subpopulations, demonstrating that even the short migrations necessary to move into different refugia did not take place at a rate sufficient to ensure mixing of the whole bin population. This study is the first to combine the interaction of refugia and incomplete application of insecticide with the evolution of insecticide resistance over generations, on a scale that is large enough to mimic those in a true grain store. Contrary to theoretical models and laboratory studies the grain bin population that survived in this study had a higher level of resistance than the source population. The level of insecticide resistance in the grain bin populations rose in just one generation. These residual populations, despite being in insecticide free areas, harbour resistant individuals, and can therefore provide a focus for continual reinfestation of grain supplies.

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18-116

ESSAYS AT APPLICATION OF THE MATHEMATICAL MODELING - THE EFFECT OF DIFFERENT AIR TEMPERATURES ON THE DURATION OF DEVELOPMENT AND THE INVASIVE ACTIVITY OF COMMON BEAN WEEVIL *ACANTHOSCELIDES OBTECTUS* SAY (COLEOPTERA, BRUCHIDAE)

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A temperature is one of the most essential factors influencing the energy flow and distribution in animal body.

In case of coleopterous common bean weevil (*Acanthoscelides obtectus* Say) the temperature change from 28°C (optimal temperature) to 20°C causes the seven times drop of female fertility and twice as longer the larvae development time.

Also the larvae invasive activity (successful settlements in bean seeds) decreases considerably.

Presented mathematical model is based on own experiments.

18-118

MITES AND INSECTS IN URBAN PIGEON'S NEST

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The pigeon (*Columba livia domestica*) is one of the gregarious species which has settled in remarkable proportions in our cities. Pigeons build their nests in the garrets of buildings, monuments and ruins. These nests constitute an ideal habitat for many insects and other arthropods.

The objective of this work was to make a census of the arthropod fauna present in the nests of domestic pigeons, which live in the bell towers and attics in the city of Como (Northern Italy), in order to evaluate eventual risks for the health of man, linked to the presence of these hosts.

The arthropod fauna found resulted very varied: 11 orders of insects have been identified. Most of the samples taken were in the larval stage and their classification did not go any further than order. Just as numerous were the arachnids (aranea, pseudoscorpions and mites). Furthermore, crustacean isopods were seen in great numbers, especially in heaps of guano.

Among the insects there did not seem to be any harmful to man.

The mites identified are not regarded as being among those potentially pathogenic to man by literature, with the exception of the pigeon tick, which causes allergies in many individuals, sometimes lethal.

18-119

A method for the detection of mites in house-dust samples

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House dust mites exposure is an important risk factor in the development of allergic syndrome. The development of methods for detection of mites in domestical environments would be helpful in planning more proper anti-mites measures. Aim of this study is to develop simple, specific and rapid test for the evaluation of mites allergens in house-dust samples.

It is based on a nitrocellulose dipstick spotted with specific anti-mite allergens antibodies (capture matrix). Such antibodies, when conjugated with colloidal dye particle acts, as detecting reagent. The assay is one-step configuration, spotted dipstick being added in a tube containing detecting reagent and house-dust sample. No instrumentation is required, the response being visible at the naked eye as a colored spot after an hour of incubation. By analysis of several HDM samples, this method seems to be more specific than commercial guanine determination test even if less sensitive than Der p I Elisa.

18-121

SENSITIVITY OF *Anagasta kühniella* (Zeller, 1879) (LEPIDOPTERA, PYRALIDAE) AND ITS NATURAL ECTOPARASITE, *Habrobracon hebetor* (Say, 1836) (HYMENOPTERA, BRACONIDAE) TO MICROWAVES (2450 Mhz)

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The Mediterranean flour moth, *Anagasta kühniella*, a cosmopolitan pyralid, is well known as one of the most important pests of stored products. Its larvae are naturally parasitized by *Habrobracon hebetor*, a very effective ectoparasite. Some physical control methods, including radio-frequencies, as microwaves, are being evaluated aiming to establish IPM programmes. The present work, therefore, was undertaken to determine the comparative effect of microwaves (2450 Mhz) on *A. kühniella* adults and larvae, as well as on *H. hebetor* adults.

Different microwave exposure times (ranging from 5 up to 34 seconds) were established. For *A. kühniella* bioassays, 100 individuals per treatment were utilized, while for the case of *H. hebetor*, 48 individuals per treatment were used. Median Lethal Exposure Time (LE50) criterium was applied; and the LE50 values were calculated 2, 12 and 18 hours after exposure. Concerning *A. kühniella*, the adults (with LE50 varied between 8 and 9 seconds) showed to be more sensitive than the larvae (with LE50 varied between 9 and 18 seconds), among the three evaluation periods. Moreover, larvae within the diet (LE50 varied between 9 and 11 seconds) revealed higher sensitivity than those directly exposed to radiation (LE50 varied between 14 and 18 seconds). *H. hebetor* adults showed to be significantly less sensitive than *A. kühniella* adults and larvae. The highest mortality (20.8 %) observed among treated braconid wasps was obtained 18 hours after an exposure time of 34 seconds. These results stimulate future investigations to verify the compatibility degree between these two mortality factors (biotic and abiotic) in IPM programmes for the control of this insect pest.

18-120

POTENTIALITIES OF MALATHION-BIFENTHRIN MIXTURES TO PROTECT STORED PRODUCE FROM PESTS

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The most common storage pests in Romania are *Sitophilus oryzae*, *S. granarius*, *Rhizopertha dominica*, *Oryzaephilus surinamensis*, *Tribolium confusum*, *Cryptolestes* sp. and *Plodia interpunctella*. In more humid areas mites are also sometimes troublesome. Psocids are often present in stores throughout the country. *Acanthoscelides obtectus* is widespread in stored haricot beans.

It was found that insect pests can reproduce within a time span extending from mid May through mid October in southern zone, while in early June-early October in the North. Winter low temperatures have little influence on storage insects and mites.

Opportunities to protect grain and beans in storage with malathion-bifenthrin mixtures (20:1) have been investigated in commercial grain silo bins, flat stores and empty storage premises. Suitable results have been obtained with rates of 6.0+0.3 mg/kg applied as protectants, and 500+25 mg/sq m. when spraying storage premises prior to grain introduction.

Malathion-bifenthrin mixture applied at a rate of 500-25 mg/sq m. was also efficient in controlling full-grown *P. interpunctella* larvae crawling on top of a grain bulk seeking for pupation sites. It is thought that UL formulations are adequate for applications in commercial stores, while EC sprays are convenient to household or on-farm storage of smaller amounts of produce.

18-122

INSECTICIDAL ACTIVITY OF PLANT ORIGIN PREPARATUS AGAINST STORED PRODUCT INSECTS

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Plants dust and oil extract of *Rosmarinus officinalis*, *Laurus nobilis*, *Thymus vulgaris*, *Origanum vulgare*, *Lavandula officinalis* were evaluated for the control of infestations *Acanthoscelides obtectus* Say. and *Sitophilus granarius* (L.) imago in laboratory conditions. All investigated plants species have insecticidal influence on *A. obtectus* and *S. granarius*.

For the control of *A. obtectus* the most effective was oil of *L. officinalis*, with dose of 0.1 ml per kg *Phaseolus vulgaris* L. (total mortality per 24 h). Less effective was dust (*L. officinalis*, *O. vulgare*) with dose of 1g per kg bean with total mortality of *A. obtectus* during 48 and 72 h exposition. Dust of *L. nobilis*, *T. vulgare* and *R. officinalis* had lower efficacy (total mortality of *A. obtectus* was attained after 15 days).

For the control of *S. granarius* in wheat - *Triticum vulgare* the most effective was dust of *L. nobilis* with dose of 1g per 1kg (total mortality during 26 days), and less effective with dust of *R. officinalis*, *T. vulgare* and *L. officinalis* during 28 and 30 days of exposition.

By the investigation in store house conditions with dust and oil extract of *R. officinalis*, *L. nobilis*, *Th. vulgaris* and *L. officinalis* against *S. granarius* in mercantile wheat, the most effective was dust of *R. officinalis* with dose of 37 g per 5 kg (total mortality per 48 h).

In seed wheat the most effective was oil extract of *L. nobilis* in dose of 3.7 ml per 5kg (total mortality after 72 h).

All investigated plant origin preparatus had not negative influence on the seed wheat germination.

18-123

EXPANSION OF SYNANTROPIC COCKROACHES SPECIES IN RUSSIA

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Of 60 species of cockroaches (*Blattoptera* order) living in the fauna of Russia only six species are synantropic ones. In general two species of synantropic cockroaches, namely *Blattella germanica* and *Blatta orientalis* are constantly registered in urban sites of the country. Nowadays fauna of synantropic cockroaches in Russia has been significantly widened due to the species delivered by various transport communication. Since 1970 some foci of *Periplaneta americana* have been registered in a number of big cities. The colonies of *Nauphoeta cinerea*, *Periplaneta australasiae*, *Leucophaeta maderae*, *Supella longipalpa* occur in the buildings of the Zoo, green houses and living places. Some cases of *Blaberus* and *Panchlora* species appearance have been fixed as well. A dynamic process of urban ecosystems settling by new species of cockroaches is being observed now. The monitoring and mapping of the insects localization in various regions of the country is carried out.

18-125

ECOTYPES OF *TRIBOLIUM* UNDER DIFFERENT FOOD CONDITIONS

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Investigations were made of the effect of altered habitat, when the animals were kept in wheat flour deprived yeast, on phenotypic differentiation in *Tribolium* strains. Fecundity, hatchability, survival and reproduction were assessed under the effect of this factor.

In *Tribolium castaneum* a considerable lowering of fecundity was observed in altered medium, whereas in *T. confusum* this factor had no effect. The altered medium did not cause changes in fecundity of ecotypes of *T. confusum* whereas in *T. castaneum* it caused lowering the fecundity almost by half. No significant effect was observed in hatchability.

Reduction of numbers was largest in the egg stage, and smallest in the pupal stage. In *T. castaneum* this reduction was smaller than in *T. confusum* and 6-instar ecotypes showed the best survival.

In the standard medium the reproductive effort was higher in *T. castaneum* than in *T. confusum*. It was also higher in 6-instar than in 7-instar ecotypes. In altered medium reproductive effort was relatively lower, especially in *T. castaneum*.

18-124

EFFECT OF TRICALCIUM PHOSPHATE ON REPRODUCTION IN *TRIBOLIUM*

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The investigations involved the influence of Tricalcium phosphate (TCP) on reproductive efficiency in two species of *Tribolium* and two ecotypes distinguished in these species. Three concentrations of TCP were used, namely 0.5%; 1.0% and 1.5%. Reproductive effort was calculated with data on fecundity, individual weight of an adult and calorific value of pupae and adults.

Increasing concentration of TCP in the culture medium caused diminishing of the body weight of adults, however not in direct proportionality.

A clear decrease in fecundity under TCP effect was observed for *T. castaneum*, and in *T. confusum* decrease is significant only at 1.0% concentration. Such decrease is lower in 7-instar than in 6-instar ecotypes.

Calorific value decreases with increasing concentration of TCP in all development stages examined, more in *T. castaneum*. No differences were observed in calorific value among ecotypes.

The reproductive effort was compared with that in control series. This index decreased in the two species, much more in *T. castaneum* than in *T. confusum*.

18-126

ADJUSTEMENT OF PHOSPHINE DOSAGES FOR CONTROL OF IMMATURE STAGES OF RESISTANT STRAINS OF *SITOPHILUS ORYZAE*

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The most important factor in order to achieve a successful fumigation with phosphine is the recognition that there exists a great difference in the tolerance to the gas among the several developmental stages of insects. To obtain information that could be used as guidelines for the adjustment of phosphine dosages to control insect resistant populations of stored products, all developmental stages of two strains of *Sitophilus oryzae* with a high frequency of resistance (as detected by the FAO discriminating dose test method n° 16), collected during a survey on resistance that covered 8 states of Brazil, were treated with doses of 1.5 and 3.5 g PH_3/m^3 and exposure periods that ranged from 72 to 336 hours, in glass dissection chambers kept in controlled environment chambers at $26 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ R.H.

For both strains and doses, exposures periods of 72 hours were not sufficient to achieve complete control, with emergence occurring as early as the second week after fumigation in cultures containing old larvae and pupae, and in the fourth week, in cultures containing eggs and young larvae. A single emergency, corresponding to a mortality rate of 99.96% in relation to the control, was observed at a CTP of $168\text{gh}/\text{m}^3$ for 120 hours exposure in the strain with the highest level of resistance (108.4 at LC 99.9). However, in a second bioassay with this strain, under the same conditions, 100% mortality was achieved within 96 hours.

Results obtained indicate that it is necessary to change label recommendations of 72 hours exposure period in commercial phosphine products sold in Brazil. However, it is unlikely that a good control would be achieved unless a considerable improvement is also introduced in current sealing practices.

18-127

COMPARATIVE EFFICACY OF DIFFERENT FORMULATIONS OF PIRIMIPHOS-METHYL AND ETRIMFOS, WHEN ADMIXED WITH WHEAT, AGAINST SUSCEPTIBLE STORAGE INSECT PESTS

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The efficacy of emulsifiable concentrate (e.c.) and dust formulations of pirimiphos-methyl and etrimfos were compared, when admixed with wheat at 0.5, 1, 2 and 4 mg kg⁻¹, against susceptible strains of *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* (L.), *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.). Immediately after treatment and then at 4 weekly intervals for a period of 36 weeks, samples were taken for pesticide residue analysis and insect bioassays. Insect mortality was assessed after 1, 7, 14, 21 and 28 days exposure. The results indicated that complete mortality of *T. castaneum*, *O. surinamensis* and *S. granarius* was achieved for 36 weeks, when exposed for at least 7 days, to recovered pesticide residues of approximately 1 mg kg⁻¹ pirimiphos-methyl e.c., 1.3 mg kg⁻¹ etrimfos e.c., 2 mg kg⁻¹ pirimiphos-methyl dust and 1.6 mg kg⁻¹ etrimfos dust. The e.c. of pirimiphos-methyl was the most effective treatment against these species. Higher mortalities were recorded at equivalent doses, and longer periods of protection were provided, with shorter exposure periods required for complete mortality. Against *R. dominica*, the dust formulation of etrimfos appeared the most effective treatment, however, complete mortality was only achieved up to 4 weeks storage. There appeared to be little degradation of pesticide residues over the experimental period although loss of bioactivity was observed.

18-129

DISPERSAL AND CONTROL OF *Aedes aegypti* (L.) IN URBAN AREAS: STUDIES WITH A RUBIDIUM MARK-RELEASE TECHNIQUE

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The mosquito *Aedes aegypti* (L.) is the principal urban vector of Chikungunya, dengue and yellow fever, viral diseases that can spread rapidly in explosive epidemics. We have developed a method for marking the eggs of this species with rubidium (Rb), a relatively rare alkali metal. A solution of 0.045M RbCl in pig's blood yields 8-14 ppb rubidium per egg laid on the first day of oviposition; eggs laid on subsequent days contain less rubidium, but are still detectable by atomic emission spectroscopy. We have used our method to monitor dispersal and to evaluate the impact of intra-domiciliary insecticide treatments. Our results provide direct evidence that oviposition activity can last several days in the field, and that eggs are distributed over an area at least 840 m in diameter. We believe that dispersal may be affected by the availability of oviposition sites. Our results indicate that the common practice for the control of *Ae. aegypti*-borne disease by "focal" treatments with insecticidal space sprays, centered on the homes of presumed or confirmed cases, are unlikely to be effective. Furthermore, attempts to reduce adult mosquito populations by eliminating larval habitats may enhance dissemination of virus by promoting wider dispersal of the vector. Our studies also confirm that intra-domiciliary treatments with insecticidal aerosols are more effective than treatments made from aircraft or road vehicles.

18-128

MONITORING OF DIFFERENT STORED-PRODUCT PESTS USING SYNTHETIC SEX PHEROMONE

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Different sex pheromone trap designs, were used to monitor the occurrence and intensity of *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae), *Ephestia cautella* (Wlk.), *E. kuehniella* Zell. and *Plodia interpunctella* (Hb) (Lepidoptera: Pyralidae), common stored-product pests.

Field trials were carried out in four different warehouses: tobacco for *L. serricorne* and almond kernel, carob and seed-corn stores for *E. cautella*, *E. kuehniella* and *P. interpunctella*.

The potential of such pest monitoring studies was evaluated as a assessment technique in order to use them, in the future, as a decision tool for pest control in stored-products.

18-130

FEEDING DETERRENT ACTIVITY OF SOME SYNTHETIC TERPENE LACTONES

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Terpene and sesquiterpene lactones isolated from plants usually exhibit specific biological activity. Many of them showed the antifeeding activity against insects.

Continuing our studies on the synthesis of compounds which can be used for insect pest population control we have obtained mono- and bicyclic terpene and bishomoterpene lactones. Some of them were synthesized in both enantiomeric forms.

The antifeeding activity of these lactones was tested on three storage pest insects: granary weevil (*Sitophilus granarius* L., adults), confused flour beetle (*Tribolium confusum* Duv., adults and larvae) and the khapra beetle (*Trogoderma granarium* Ev., larvae). Some of lactones tested showed interesting activity.

18-131

THE POPULATION DYNAMICS OF STORED PRODUCT LEPIDOPTERA INSECTS IN REPUBLIC OF GEORGIA

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The complex of Lepidoptera insects: Nemapogon granellus L. (Tineidae), Sitotroga cerealella Oliv. (Gelechiidae), Plodia interpunctella Hb., Pyrallis farinalis L., Ephestia kühniella Zll. (Pyralidae) cause great damage to cereals in granaries. In connection with the changes in grain storage technology in Georgia during the last years, the pests are found mainly on the primary processing products - flour and groats. Bacterial and virus diseases, at a high density of larvae, both occur in mill and laboratory conditions. The study illustrates the importance of natural enemies, which determinates of the growth of population rates of several kinds of stored product Lepidoptera insects.

18-132

EVALUATION OF INSECT GROWTH REGULATORS AND THEIR MIXTURES FOR SUPPRESSION OF SYNANTROPIC COCKROACHES POPULATION

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In urban conditions sex and age composition of synantropic cockroaches can be greatly varied. That's why elaboration of alimental toxic baits for synantropic cockroaches (TBC) is of great importance.

A number of a new TBC recipes have been prepared on the basis of a mixture of insect growth regulators containing juvenil-hormon analogue (hydropren), chitin synthesis inhibitor (dimihlin) and permithrin. The efficiency of the preparations was evaluated on *Blatella germanica* and *Periplaneta americana* species by free group feeding in laboratory conditions. On the first stage a gradual death of juvenile larvae of *Blatella germanica* species was observed during ten-thirty days period that was attributed to a toxic action of dimihlin. Then an increase of the amount of chimeric insects has been observed up to 90% by the end of 30 days exposition. Feeding of the baits containing permethrin and hydropren has led to the death of all *Blatella germanica* and *Periplaneta americana* imago by the 7-th and 20-th days respectively. More than 60% of the larvae of both species were killed by the insecticides. Anomalous growth of the survived larvae has been observed by the end of 30 days period. The efficiency of the preparations was also evaluated on a number of Moscow objects occupied by *Blatella germanica* species. Suppression of the insect population was observed during 2-3 months period. A very low toxicity level for a human was also demonstrated.

Section 19

Ecology of Pesticides,
Resistance and Toxicology

19-002

Patterns of evolution and spread of mosquito resistance suggested from comparative analysis of the EST α 2/EST β 2 and EST β 1 amplicons.

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The major insecticide resistance mechanism in *Culex quinquefasciatus* mosquitoes involves two amplified esterases. More than 90% of resistant field populations have a single amplicon on which two esterases, Est α 2 and Est β 2 occur. At a molecular level these esterases are identical in field populations from different sources. In contrast the remainder of the resistant populations, with one exception, contain two esterases, Est β 1 and Est α 3. These esterases are, however, on separate amplicons. At a molecular level the Est β 1 genes differ between the 3 populations analysed.

The gene sequence and structure of the Esterases suggests that the Est α s and Est β s arose originally from gene duplication. They also remain tightly linked in susceptible insects.

This paper will discuss the origin of the different amplicons. Evidence will also be presented to suggest that the fitness of individuals carrying the Est α 2/Est β 2 amplicon may be profoundly influenced by the presence of a third non-esterase gene within the amplicon. This may explain why this amplicon has spread so rapidly, despite the lack of an obvious fitness advantage of the Est α 2 and Est β 2 over the other esterases with regard to resistance confirmation.

19-001

EVOLUTION OF INSECTICIDE RESISTANCE IN THE
MOSQUITO *CULEX PIPPIENS*

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The evolution of insecticide resistance in the mosquito *Culex pipiens* can be studied at the gene level, due to the recent progress of molecular tools. Esterase overproduction is a common resistance mechanism to organophosphate insecticides, and two esterase genes are involved: *esterase A* and *esterase B*. Using extensive population data (from five continents, from treated and untreated areas), analyzed for both loci at the protein and DNA level (RFLP, sequence), it is concluded that mutation conferring resistance have occurred only few times at these loci, but have spread worldwide since their first occurrence. This limitation of mutation and the importance of migration are discussed.

19-003

WHAT DO WE KNOW ABOUT THE ORIGIN OF
AMPLIFIED ESTERASE GENES IN *MYZUS PERSICAE*?

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Insecticide resistance in the aphids, *Myzus persicae* and *Myzus nicotianae* is conferred by the presence in both species of amplified E4 or FE4 genes encoding two closely related insecticide-detoxifying esterases. The FE4 gene is amplified in aphids of normal karyotype, and E4 in aphids with an A1,3 chromosomal translocation. The two forms of amplified gene are very similar, with the same exon/intron arrangement, only 20bp differences in their cDNA and a high nucleotide conservation within intron sequences. So what is the origin of these amplified genes?

Although data from restriction mapping of the esterase amplicons is consistent with single origins of each amplification (one for E4 and one for FE4) as has been suggested in *Culex* mosquitoes, this cannot be confirmed by mapping of susceptible aphid DNA. However, spread between populations is clearly occurring since both *M. persicae* and its tobacco-feeding form, *M. nicotianae*, from around the world have the same amplified genes. Sequencing of esterase genes has shown that E4, FE4 and at least two other closely related sequences are present in all individuals although only E4 or FE4 is amplified, and *in situ* hybridisation shows that the amplified genes can occur at several loci (even within the same aphid). This all points to *M. persicae* having a family of esterase genes, two of which have amplified once or more and then spread both around the aphid genome and throughout aphid populations worldwide.

19-004

ORIGIN AND SPREAD OF CYCLODIENE INSECTICIDE RESISTANCE ASSOCIATED WITH POINT MUTATIONS IN A GABA RECEPTOR GENE

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The number of independent origins of insecticide resistance alleles is currently the subject of intense debate. Support for the importance of a single point of origin and spread of resistance through insect populations comes from studies of amplified esterases and insensitive acetylcholinesterase in *Culex* mosquitoes. Here we argue that it is difficult to determine precisely the number of origins of resistance alleles due to the complexity of the two mechanisms studied in *Culex*. The repeated replacement of the same amino acid in the *Resistance to dieldrin (Rdl)* gene, conferring resistance to cyclodiene insecticides, offers a model system within which to examine the diversity and origins of resistance alleles. By comparing *Rdl* alleles in two *Drosophila* species, two beetle species and the *Bemisia tabaci* whitefly complex we present repeated evidence for multiple independent origins of resistance. Evidence for independent origins comes not only from the finding of different replacements of this same amino acid but also flanking sequence data supporting multiple origins of the same amino acid replacement. Further, we emphasize that the life history of the insect under consideration can play a major role in determining the likely origin and spread of different resistance alleles.

19-006

ESTERASE ALLOZYME AS ELECTROPHORETIC MARKER FOR RESISTANT MECHANISM OF *Plutella xylostella* (L.) (LEPIDOPTERA:PLUTELLIDAE)

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Thirteen esterase loci were estimated from the PAGE zymogram of *Plutella xylostella* (L.) (DBM) of Taiwan. Two loci, *Est 8* and *Est 9*, were easily coded in the 17 local DBM populations. *Est 8* has four alleles: *a*, *b*, *c*, *null*, and *Est 9* has three alleles: *a*, *b*, and *null*. All these allozymes were susceptible to 10^{-6} M paraoxon except *Est 9^b*. Two strains were established from the population of Sheh-Tze(ST), Taipei, through more than 6 generations of sib-mating. The malathion-resistant strain, ST10, selected through single female-male pairing method with malathion as selection force was bestowed with high frequencies of *Est 8^b* and *Est 9^b*. The susceptible strain, ST12 was bestowed with high frequencies of *Est 8^a* and *Est 9^a*. The ST10 insect had higher enzymatic activities of monooxygenase, glutathione-S-transferase, and acetylcholinesterases than that of ST12. Besides, acetylcholinesterases of ST10 was more tolerant to 10^{-4} M eserine than that of ST12.

Est 9^a of ST12 was stable and would not disappear from the zymogram of the larva during the investigation of consequent 6-generation sib-mating, and *Est 9^b* of ST10 was unstable and would disappear during the sibmating experiment, especially when malathion was applied as a selected pressure to the DBM; these phenomena hinted that regulatory factor for esterase allozymes was possibly associated with the resistance mechanism.

19-005

MULTIPLE SITES OF THE AMPLIFIED ESTERASE GENES THAT CONFER RESISTANCE TO INSECTICIDES IN *MYZUS PERSICAE* (SULZER) (HOMOPTERA: APHIDIDAE) REVEALED BY FLUORESCENCE *IN SITU* HYBRIDISATION

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Myzus persicae combats organophosphorus and carbamate insecticides by overproducing insecticide-degrading esterases, encoded by amplified genes. Resistant aphids have one of two alternative amplified esterase genes, E4 or FE4, depending on their karyotype. Aphids with an autosomal 1,3 translocation have E4 genes, whereas those without this translocation have FE4. We have used fluorescence *in situ* hybridisation (FISH) to locate the amplified esterase genes in mitotic and meiotic chromosomes of resistant aphids. Specific binding to amplified E4 and FE4 sites was achieved with a genomic probe encompassing most of the esterase gene. Chromosomal locations were confirmed by inheritance studies and by pairing relationships in male meiosis I, and the esterase type of each clone was diagnosed by Southern blotting. Whereas E4-producing clones usually have all their E4 sequences at a single site closely linked to the A1,3 translocation, FE4-producing clones can have several loci which bind to the probe on autosomes 1, 2 and/or 3, and can be either heterozygous or homozygous for amplified FE4. Overall, amplified E4 and/or FE4 sequences were found on 4 of the 5 autosome pairs of *M. persicae*. The evolution of both E4- and FE4-based resistance, including the role of the translocation and possible origins of the multiple loci, will be discussed.

19-007

ANALYSIS OF SODIUM CHANNEL GENE SEQUENCES IN KDR & SUPER-KDR HOUSEFLIES (*MUSCA DOMESTICA*)

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The pyrethroids are a major class of synthetic insecticides with widespread uses in the control of agricultural and human health insect pests. Their intensive use over the last 20 years has, however, led to the development of resistance in many insect species, and this represents a significant and increasing threat to their continued effective use. An important mechanism of resistance to pyrethroids, known as knockdown resistance or *kdr*, confers nerve insensitivity to these compounds and is thought to result from a modification of their primary target site, the voltage-sensitive sodium channel. This type of resistance has been best characterised in the housefly where several *kdr* alleles, including the more potent *super-kdr* factor, have been identified.

To investigate the molecular basis of the *kdr* mechanism, we have cloned the full 6.3kb coding sequence of the *para*-type sodium channel gene from a susceptible housefly strain. The cDNAs predict a polypeptide of 2108 amino acids with close sequence homology (92% identity) to the *Drosophila para* sodium channel, and around 50% homology to vertebrate sodium channels. Comparative sequence analysis of a range of housefly strains carrying *kdr* and *super-kdr* resistance factors has revealed two amino acid mutations that correlate with the resistance phenotypes. Both mutations are located in a region of the sodium channel known to be important for channel inactivation.

19-008

SEQUENCING OF DNA STRUCTURES OF THE PARA-TYPE SODIUM CHANNEL GENES FROM KDR-RESISTANT *BLATTELLA GERMANICA* AND *MUSCA DOMESTICA*

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Using reverse transcription polymerase chain reactions (RT-PCR), the DNA sequence for the main membrane spanning region (IS3, through 20 bases beyond IVS6) of the gene encoding the α -subunit of the para-sodium channel of the German cockroach, *Blattella germanica* has been identified. The overall structure of this open reading frame region of the *Blattella germanica* gene is very similar to the para gene of *Drosophila melanogaster*, and that of the partially sequenced para gene of *Musca domestica*. On the other hand, it is distinctly different from that of the DSC gene (*Drosophila* sodium channel). As a result of a side-by-side comparison for the para gene sequences of the susceptible CSMA strain against the *kdr* resistant VT strain of *B. germanica*, one mutation (TTG to TTC) at the approximate center of the IIS6 membrane spanning region was found to result in an amino acid shift from L to F. While functional meaning of this mutation on the operation of the para-sodium channel remains to be studied in the future, it has been noted that this region is very conserved among all sodium channels identified so far in many species and is the most hydrophobic area of the entire α -subunit. For comparison, we have studied the same region of the para-sodium channel of both *kdr* and susceptible houseflies, *M. domestica*. We found the similar type of mutation, CTT to TTC, resulting in the same shift of amino acid L to F on this site. However, in the case of houseflies, both *kdr* and susceptible strain contained L and F.

19-010

NONSTEROIDAL ECDYSTEROID AGONISTS AS INSECT CONTROL AGENTS

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The diacylhydrazine ecdysteroid agonists were first discovered by Rohm and Haas scientists over ten years ago. Extensive research in this area has so far yielded one commercial insecticide, tebufenozide, and two promising compounds in the pre-commercialization phase, RH-2485 and RH-0345.

Tebufenozide (MIMIC™, RH-5992) is a safe, effective, and highly selective caterpillar control agent for use in a wide range of vegetable, orchard, and agronomic crops. Because of its extraordinary safety to a wide range of predatory and parasitic arthropods, it has proven to be an effective tool for integrated pest management.

RH-2485 is a second generation caterpillar control agent. It is significantly more potent than tebufenozide at both the target site and the whole insect level. It has a broader caterpillar control spectrum.

RH-0345, currently under joint development in the US with American Cyanamid Corporation, is a highly effective soil/foliar applied control agent for grubs and caterpillars in turf.

The history, mode of action, and insecticidal properties of these compounds will be discussed.

19-009

SPINOSAD, THE FIRST MEMBER OF A NEW CLASS OF INSECT CONTROL PRODUCTS, THE NATURALYTES
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Spinosad, the first product in the naturalyte class of insect management tools, is a mixture of two fermentation-derived products produced by *Saccharopolyspora spinosa*, a new species of Actinomycete discovered in a Virgin Islands rum still. This material exhibits such a favorable environmental and mammalian toxicity profile that it has been designated a reduced risk pesticide by the United States EPA. Spinosad is rapidly degraded in light, but is stabilized on leaf surfaces. Its rainfastness as a suspension concentrate is comparable to commercial standards, and its activity is unaffected over a wide range of pH's. Spinosad is selectively active on insects in the Orders: Lepidoptera, Diptera, Thysanoptera, and some Coleoptera and Hymenoptera. Targeted crops are cotton, vegetables, tree fruits, and nuts at use rates of from 50-180 grams AI/Ha. Spinosad, with its unique characteristics, does indeed fit a class of its own, and will offer an exciting pest management alternative for the future. No other naturally sourced material has its' combination of excellent contact and residual efficacy on target pests and safety to beneficials, aquatic organisms, and mammals. It will fit very well into Insect Pest Management Systems.

19-011

PYRROLE INSECTICIDES: DISCOVERY, PHARMACODYNAMICS AND BIOLOGICAL ACTIVITY

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Following isolation and characterization of dioxapyrrolomycin (an actinomycete fermentation product), chemical synthesis work was started at American Cyanamid to obtain a highly active insecticide of novel structure and action mechanism. *In vitro* mitochondrial assays demonstrated that these synthetic pyrroles exert their insecticidal impact via uncoupling of oxidative phosphorylation. Structure-bioactivity relationships indicated that pesticidal properties of this chemical series could be optimized via differential pyrrole ring substitutions (e.g., electron withdrawing groups). Analogs possessing a balance of molecular acidity and lipophilicity in a pKa range of 7.0 - 7.8 and logP of approx 5.0 demonstrated greatest insecticidal activity. An N-substituted 2-arylpyrrole, CL 303,630, is known to be a prodrug and possesses excellent insecticidal and acaricidal activity (it is currently in a global registration and development program).

19-012

PYMETROZINE, AN EFFICIENT SYNTHETIC FEEDING INHIBITOR SPECIFIC TO HOMOPTEROUS PESTS, IS TRANSLOCATED IN BOTH PLANT XYLEM AND PHLOEM.

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Pyrimethrin (CGA 215'944) is a highly selective insecticide against plant sucking pests, provoking an immediate feeding stop and a subsequent starvation of affected insects to death. High efficacy against target pests is achieved after both drench and foliar application on plants.

Acropetal translocation of the compound is evident with the protection of the shoot against aphids after drench application, and can be explained by xylem transport. Additionally, systemic effects were also observed after foliar application. With a sequence of bioassays it could be shown that activity resulted not only from an acropetal but also from a basipetal translocation of the compound. This translocation is typical for photoassimilates, which move from source (mainly expanded leaves) to sink (roots, growing points and emerging leaves) over the phloem. After foliar application, pyrimethrin translocation in the phloem may therefore contribute to the long term control of aphids and whiteflies, since it is imported from the leaves to the growing parts of the shoot. This translocation pattern of the compound in the plant, which has been inferred from bioassays, was confirmed by detection and visualizing of pyrimethrin using autoradiographic techniques.

19-014

NEONICOTINOIDS-RETROSPECT AND PROSPECT

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Nithiazine was invented as the first nitromethylene insecticide, though not commercialized. However, introduction of the 3-pyridylmethyl group on nitromethylene heterocycles remarkably increased the insecticidal activity and further optimization resulted in the invention of imidacloprid. It was shown that nithiazine and imidacloprid are agonists to the nicotinic acetylcholine receptor (nAChR), and imidacloprid and nicotine shared the same structural moiety, structure-activity relationships and mode of action. Because of the invention of several analogs of imidacloprid, which have either nitromethylene, nitroimino or cyanoimino group, they can be collectively called neonicotinoids. However, there are distinct differences between neonicotinoids and neonicotinids. Nicotine is highly toxic to mammals and has limited insecticidal activities, while imidacloprid has the opposite properties. Nicotine should have a basic nitrogen atom, which is positively charged in the insect body and provides a site of interaction with the nAChR, while the corresponding nitrogen atom in imidacloprid is not ionized but bears a partial positive charge, which is enough to interact with the insect nAChR, but not enough with the vertebrate one. Novel neonicotinoids, nitenpyram and acetamiprid share the above characteristic, but differ from imidacloprid in stability and insecticidal spectrum, thus can be complementarily utilized. The hydrophobicity of the compounds is a key factor. These generalization provide a biorational basis for further development of this series.

19-013

NEW ANTI-INSECT EXTRACT FROM A FOLK PLANT IN SOUTH AMERICA (VALLESIA GLABRA-APOCYNACEA).
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Vallesia glabra is the latin name for Ancoche which is the vernacular name for a ten foot tall bush that belongs to the Apocynacea family. Ancoche can be found from Florida up to Argentina, overlapping the endemic area of Chagas' disease in the American continent. Triatoma infestans is the latin name for Vinchuca which is the major vector of Chagas' disease or American Trypanosomiasis in Argentina. The disease was first described by Carlos Chagas in 1909 and still remains endemic and incurable in 1996. Ancoche was first described in 1799 but has never been tested as a potential insect control agent. In pursuing the study of anti-triatoma properties of extracts prepared from indigenous plants in Argentina, it was assessed the in vivo activity of Ancoche in vinchucas. Films or topical application bioassays showed acute toxicity in nymphs and adults. In fact, 5 ug of purified fraction per insect showed 100% mortality. The acetonic extracts did not show any repellency, but in survival females exposed to Ancoche extracts produced a significant decrease in laying eggs, and also a much lower ratio of hatched eggs. The purified fraction from Ancoche extracts showed anti-cholinesterase activity (IC₅₀=15 ug/ml) and also inhibition of glutathione S-transferase from T. infestans (IC₅₀=50 ug/ml). This preliminary study is the first concerning a botanical material active against the major vector of Chagas' disease in South America.

19-015

Imidacloprid: Discovery and Development

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Imidacloprid, [1-(6-chloronicotinyl)]-2-nitroiminoimidazolidine, is a new type of insecticide possessing potential activity and novel modes of action. It controls resistant insects, has a broad spectrum of activity especially against sucking pests, excellent systemic properties and high residual activity. Imidacloprid was the end product of about 2000 compounds prepared during the synthetic study started by modifying the structure of the lead compound, nithiazin. The key step to the development of the new pesticide class was finding of a latent activator, 6-chloronicotinyl group. By introducing this group to the skeletons of the leading molecules the original insecticidal activities were increased enormously by a factor of several hundreds, and the target insect sorts were remarkably broadened. There remained dozens of equally active candidates for commercial use at the laboratory test levels. Imidacloprid was selected among them, principally owing to its superior properties under practical conditions. It is rather hydrophobic among the generally hydrophilic compounds in this class, is stable under acidic and neutral conditions, and resists air-oxidation. The definitive advantage of imidacloprid over the nitromethylene analogues like nithiazin is its photostability. The nitromethylene compounds decompose rapidly under the sunlight because of the chromophore of longer wavelengths than 290 nm.

19-016

FIPRONIL, AS A NEW BROAD SPECTRUM INSECTICIDE WHICH ACTS ON THE GABA REGULATED CHLORIDE CHANNEL.

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The new insecticide, fipronil, represents a new class of chemistry with significant broad spectrum insect activity. The initial lead to this class of chemistry was discovered at the Rhône Poulenc Agro Sector research facilities at Ongar, England and was further developed into a commercial product at the R&D center at the Research Triangle Park, US. The initial compound was tested as a herbicide lead which turned out to have weak insecticide activity. This hint of activity was then the focus of a multi-year synthesis program that eventually led to the definition of the class of chemistry presently referred to as phenylpyrazoles.

These phenylpyrazoles, exemplified by fipronil, have been shown to act primarily on the GABA regulated chloride channel located in the central nervous system of the insect. This is supported by binding data on housefly tissue and by *Xenopus* oocyte data generated on a homomultimer of an insect GABA gene known as Rdl. There is also evidence that the inhibition of the GABA receptor is somewhat selective when one compares binding in vertebrate versus invertebrate tissue. Expression of the *Drosophila* Rdl in whole insect cells also gives a functional receptor that has pharmacological properties similar to that of the natural receptor that is presumed to be heteromeric.

Fipronil has been shown to be very much a broad spectrum product with especially good activity on certain insects, such as housefly, locust, and mole cricket. The biological activity is a mixture of topical and ingestion depending on the insect and the method of application. Field data has been consistent and has led to the registration of the active ingredient in a number of countries and crops around the world.

19-018

NOVALURON (MCW-275), A NOVEL BENZOYLPHENYL UREA, SUPPRESSING DEVELOPING STAGES OF LEPIDOPTERAN, WHITEFLY AND AGROMYZID LEAFMINER PESTS.

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Novaluron, 1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl) urea, a novel benzoylphenyl urea, acts by inhibiting chitin formation, thereby causing abnormal endocuticular deposition and abortive molting. While most of benzoylphenyl ureas act mainly by ingestion, Novaluron seems to act by both ingestion and contact. As such it is a powerful suppresser of lepidopteran larvae such as *Spodoptera littoralis* and *Helicoverpa armigera* (by ingestion) and of the cotton whitefly larvae, *Bemisia tabaci* (by contact). The compound is now under advanced development by Makhteshim Chemicals.

The LC₅₀ value of Novaluron on 3rd-instar *S. littoralis* fed on castor bean leaves treated with the compound is ~ 0.1 mg (AI)/liter. This value resembles that of chlorfluazuron and is about 10-fold lower than that of teflubenzuron. An application of 250 g (AI)/ha in cotton fields resulted in 100% mortality of both *Spodoptera* and *Helicoverpa* larvae up to day 8 after application and about 60 and 30% mortality, respectively at day 15.

Novaluron affects, to a much higher extent, eggs and larvae of *Bemisia tabaci* than chlorfluazuron and teflubenzuron. Suppression of 100% egg hatch was obtained at a dipping concentration of 0.5 mg (AI)/liter and of 1st and 2nd instars at a concentration of 1 mg (AI)/liter.

Novaluron is far more active in suppressing developing stages of the leafminer *Liriomyza huidobrensis* than teflubenzuron and chlorfluazuron. Suppression of over 80% adult formation was obtained at a concentration of 0.8 mg(AI)/liter and a similar suppression of pupation and mine formation at a concentration of 20 mg (AI)/liter. Addition of 0.1% surfactant to Novaluron solution, increased considerably its potency, resulting in 80% and 100% suppression of pupation and adult formation, respectively, at a concentration of 0.8 mg (AI)/liter.

Our results indicate that Novaluron is a potential compound for controlling Lepidopteran pests, whiteflies and agromyzid leafminers in field crops, vegetables and ornamentals.

19-017

MOLECULAR MODELING OF OCTOPAMINERGIC AGONISTS

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Crowding, cold shock, and heat stress, which did not affect octopamine (OA) titers, as well as juvenile hormone (JH)-esterase inhibitor delayed pupation of *Tribolium freemani* larvae, reducing JH-esterase activity. Isolation, light irradiation, vibration, which stimulated OA titers, and OA agonists as well as precocene accelerated the pupation, increasing JH esterase activity. Thus OA agonists may be useful as insect growth regulators.

The quantitative relationship between the structure of 2-(substituted benzylamino)-2-thiazolines (SBTs) and their OA-agonist activities against the nervous tissue of American cockroach *Periplaneta americana* was studied by using reported physicochemical parameters and regression analysis. The electronic feature of a substituent was important: the more electron-donating the substituent, the greater the activity against *P. americana*.

Molecules were built by using the BIOSYM package of programs. Molecular geometries of elonidine, (R)-OA, and the formamidic insecticide, chlordimeform, were obtained from available X-ray crystallographic data and were used as the starting point for subsequent calculations. Each structure was energy-minimized by using MOPAC program (6.0) running on the Cray super computer system (Y-MP2E/264) and then reoriented to compare the superimposition of the phenyl group and side-chain N atom of each structure: these features were postulated to be important for OA activity. Based on the comparison of OA and known OA agonists, it was proposed that new compounds could be synthesized which possess similar structural features to OA and OA agonists.

19-019

LOCUST CONTROL TREATMENT CONDITIONS USING FIPRONIL : EXPERIMENTAL RESULTS.

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Fipronil, a phenyl-pyrazol pesticide, was developed by Rhône-Poulenc research scientists in 1981. This pesticide compound acts by inhibiting the GABA system involved in nerve signal transmission in insects.

Vegetable oil-based treatment formulations were prepared and meet acaricide specification criteria. The LD₅₀ and LD₉₀ of the product were determined through laboratory tests against several acridians, i.e. *Schistocerca gregaria* (Forskål, 1775), *Locusta migratoria* (L., 1758) and *Locustana pardalina* (Walker, 1870). The means of contamination and immediate and delayed effects were specified for 4 and 8 g a.i./ha doses under seminatural conditions (field cages) with Desert Locusts and Migratory Locusts. In operational conditions (total coverage), tests were carried out to perfect practical aspects of fipronil control treatments against Desert Locusts and various grasshoppers, and to assess fipronil effects on some non-target fauna.

The results showed that fipronil is an efficient acaricide at very low doses (4-8 g a.i./ha). It acts on contact (knockdown effect) and ingestion (delayed effect), which additively leads to very high locust mortality (>98%) within a few days. When tested at 11 g a.i./ha, fipronil remained toxic to locusts for 3-4 weeks. It could therefore be used in barrier treatments, essential for preventive locust control, especially against Desert Locusts.

19-020

EFFECTS OF A NONSTEROIDAL MIMIC OF 20-HYDROXY-ECDYSONE ON SURVIVAL AND DEVELOPMENT OF THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.)

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Effects of a 20-hydroxyecdysone mimic, viz., RH-5992, on various stages of the diamondback moth, *Plutella xylostella* (L.), were investigated by feeding larvae on the kale leaves dipped into different concentrations of the mimic. This compound was effective to cause larval mortality only when treated various instars with the concentrations over 400 ppm. Although almost 100% cumulative mortality was obtained by treating 1st through 3rd instars with 400 ppm, the 4th instar was slightly lowered down to 87.5% with 400 ppm but maintained 97.5% with 800 ppm. The LC₅₀ values were 96.3, 95.3, and 105.8 ppm for 1st, 2nd, and 3rd instars, respectively. The LT₅₀ of 4th instar was ranged from 3.4 to 1.7 days at 100 to 800 ppm while it was ranged from 2.3 to 12.3 days for the other instars. The leaf consumption per larva was lower in the treatments with 100 ppm or above than the untreated control. The pupal weight, percent emergence, adult longevity and fecundity were affected when newly hatched larvae were continuously reared on the leaves treated with different concentrations of RH5992.

19-021

REDUCTION IN SPRAY VOLUMES AND PESTICIDE APPLICATION RATES IN POME FRUITS THROUGH IMPROVEMENTS IN SPRAY TECHNOLOGY.

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The control of pests and diseases of pome fruits has commonly depended on high volume (1000-2000 litres per hectare) spraying. A large scale trial conducted in four Tasmanian apple orchards over two seasons demonstrated spray volumes can be successfully reduced to as little as 55 litres per hectare by optimising droplet diameter to 100 microns using Micon X1 spinning disks fitted to standard airblast sprayers.

Pesticide rates as low as 25% of the label rate achieved commercially acceptable control even under high levels of pest and disease pressure. The trials demonstrated the cost of spray application could be reduced by about 50%, largely due to time savings, which had the benefit of also achieving greater timeliness of application when the periods for spraying were limited by adverse weather. The commercial acceptance of even 25% of the label rates suggests integrated pest management programs that reduce insect, mite and disease pressure in apples to low levels prior to periods that favour pest outbreaks will allow the use of considerably less than label rates in many instances.

Very low volume applications were also successful in hops and glasshouse crops.

This investigation adds to the growing weight of evidence that suggests legislative restrictions that disallow the use of less than label rates and/or very low volumes are unnecessarily inhibiting the desire to reduce the use of pesticides and reduce the costs of pest management.

19-022

ACTION OF A NOVEL NONSTEROIDAL ECDYSTEROID AGONIST TEBUFENOZIDE IN *CYDIA POMONELLA* LARVAE IS RELATED TO PREMATURE MOULTING AND ARREST OF FEEDING: AN ULTRASTRUCTURAL ANALYSISE. Viñuela¹, G. Smagghe², F. Budia¹, J. Avilla³, D. Degheele²

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Toxicity assays indicated the substituted dibenzoylhydrazine tebufenozide (RH-5992) to be a potent agonist of 20-OH ecdysone, the natural insect moulting hormone, when tested on third-instar larvae of the codling moth, *Cydia pomonella* (L.). Upon ingestion, larvae showed characteristic effects of precocious larval moulting within 12 h of treatment, leading to head capsule apolysis and cessation of feeding. Shortly afterwards, intoxicated larvae died in a double cuticle. Phenotypic and structural changes of the integument related to new cuticle formation, and of muscle structure and attachment were studied by light, scanning and transmission electron microscopic analysis of control and treated preparations. This compound appears to act specifically on the ecdysteroid receptor of the epidermal cells and is suitable for use in crop protection due to its novel chemistry and mode of action and high selective toxicity for Lepidoptera.

19-023

COMPARATIVE TOXICITY OF FOLIAR AND SYSTEMIC APPLICATIONS OF TWO NICOTINYL INSECTICIDES (IMIDACLOPRID AND ACETMIPRID) AGAINST THE WHITEFLY *BEMISIA TABACI*
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Comparative bioassays of two nicotinyl insecticides, imidacloprid and acetmiprid, against the whitefly *Bemisia tabaci* (Gennadius), using foliar and systemic applications, were conducted under controlled room conditions, and field trials using foliar applications against aphids and whiteflies were done in a cotton field. Under controlled conditions, the ovicidal activity of foliar applications of acetmiprid on cotton seedlings was much higher than that of imidacloprid. According to LC₅₀ and LC₉₀ values, acetmiprid was 11- and 19-fold more potent than imidacloprid. Both compounds were effective when applied to soil against whitefly adults, however the potency of imidacloprid was somewhat higher than that of acetmiprid resulting in a LC₅₀ value of 3.6 mg (AI)/liter as compared with 10.4 mg (AI)/liter for acetmiprid. In an experimental cotton field, set in random blocks with 4 replicates, the efficacy of foliar applications of 60 g (AI)/ha acetmiprid and 210 g (AI)/ha imidacloprid was compared. Acetmiprid was very effective against *Aphis gossypii* Glover populations for 24 d after treatment, while imidacloprid was somewhat less effective. Residual activity of acetmiprid on whitefly adults lasted for approximately 10 d and of imidacloprid for only 3 d. Our results indicate that both nicotinyls are potential compounds for controlling whiteflies and aphids either by foliar or systemic application in field crops, vegetables and ornaments.

19-025

RH-0345, A NOVEL INSECT GROWTH REGULATOR: IMPACT OF SOIL TYPE, IRRIGATION, APPLICATION TIMING, AND APPLICATION RATE ON EFFICACY AGAINST JAPANESE BEETLE, *POPILLIA JAPONICA* NEWMAN (COLEOPTERA: SCARABEIDAE) GRUBS³
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RH-0345 mimics the action of a natural insect hormone which induces the mortality and metamorphosis process in insects. This compound possesses systemic activity which could be used to simultaneously control both root-feeding and foliar-feeding pests of crops such as turfgrass. The results of laboratory, greenhouse, and field experiments studying the influence of soil type, time of insecticide application, irrigation and rainfall, and application rate on the control of several turfgrass insect pests including the Japanese beetle grubs will be discussed.

19-024

PHOTOINSECTICIDES, A NOVEL CLASS OF ENVIRONMENTALLY FRIENDLY AGENTS AGAINST *BACTROCERA OLEAE* AND *CERATITIS CAPITATA*

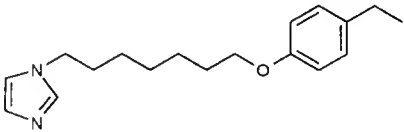
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We tested the potential of porphyrin -type photoactivatable compounds (photosensitizers) to promote the lethality of fruits flies upon exposure of the photosensitizer fed insects to sunlight .To ward this aim ,the following problems need to be addressed :
1) Choice of the photosensitizer .
2) Choice of light source
3) Definition of the minimal efficient amount of the photosensitizer
4) Definition of the optimal time of exposure to sunlight
5) Definition of most efficient light dose
For the second point we choose a halogen lamp whose emission spectrum covers the near UV/ visible range wave length and is very similar with the emission spectrum of sun
As regard the choice of the sensitizers the following parametrs were considered :
* The sensitizer must absorb spectrum with a high efficiency the largest possible portion of visible
* The sensitizer must generate toxic species such as radicals and singlet oxygen , with a high efficiency
To establish an efficient concentration of photosensitizers we adopted two methods :
a) The test of accumulation : the flies were fed with a mixture of water ,sugar and different concentrations of photosensitizer, a different concentration was used for ech group of flies
Subsequently we proceeded to the extraction of the photosensitizers from flies and to the determination of the extracted amount by emission fluorescence techniques
b) Different groups of flies that received no photosensitizer or were fed with increasing amounts of photosensitizer were irradiated under identical experimental conditions
The percent survival of flies yields the efficiency of the given photosensitizer concentration.
Analogously the efficiency of the exposure time and light power can be deduced from the percent survival .
Irradiation with natural sunlight confirms our results obtained with the artificial light source .

19-026

PRECOCIOUS METAMORPHOSIS-INDUCING ACTIVITY OF 1-SUBSTITUTED IMIDAZOLES

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We have already reported that a large number of 1,5-disubstituted imidazoles induce precocious metamorphosis in larvae of the silkworm, *Bombyx mori*. Recently we have found that various 1-substituted imidazoles induce precocious metamorphosis as well. 1-dodecylimidazole, 1-[3-(4-propylphenoxy)propyl]imidazole (**1**) and 1-[7-(4-ethylphenoxy)heptyl]imidazole (**2**) showed considerably higher activity than the other analogs containing variations in the carbon chain. Precocious metamorphosis induced by 1,5-disubstituted imidazoles, 1-dodecylimidazole and compound **1** was completely prevented by a simultaneous application of a small amount of tebufenozide, an ecdysteroid agonist, while the activity of compound **2** could not be counteracted by tebufenozide, indicating that the mode of action of compound **3** is different from that of other imidazoles.



2

19-027

FIELD EFFICACY TEST OF NEW INSECTICIDES AS TERMITICIDES

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In the course of our studies on termiticides Effects of molybdenum and tungsten compounds on termites were investigated.

These compounds possess the following characteristics (i) very slow acting and (ii) non repellent. Properties of Mo / W compound that make it suitable for use in bait block type termiticide.

We also have developed a useful method of forming waterinsoluble molybdenum and tungsten compounds. Baits prepared according to this method were applied in semi-field test in Japan and field test in Guangdong China.

The results of semifield and field tests are discussed.

19-029

PYMETROZINE: A CASE OF PERFECT SELECTIVITY

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Pymetrozine is the first representative of a new chemical class, the pyridine azomethines. It is active against sucking pests, where there is an urgent need for new compounds due to serious resistance problems. Another desirable feature is a high degree of selectivity. In three laboratory tests pymetrozine proved to be fully selective against the ladybeetle *Coccinella septempunctata*, the flower bugs *Orius majusculus* and *O. insidiosus*, and the green lacewing *Chrysoperla carnea*. In another trial, pymetrozine did not affect the parasitization capacity of *Encarsia formosa* against its host, the whitefly *Trialeurodes vaporariorum*, on tomatoes in a glasshouse when it was applied a single time.

Field selectivity trials were done on cotton, vegetables, pome fruit trees and glasshouse crops. A single spray on cotton in Egypt demonstrated excellent survival rates of the relevant predator groups (*Orius*, *Coccinella*, *Scymnus*, *Paederus*, *Chrysoperla*, spiders), comparable to the untreated control. A single spray against aphids on okra in Egypt resulted in perfect control of these pests, and surviving predators (*Orius*, *Chrysoperla*, *Coccinella*, syrphids) inhibited a reappearance of the aphid population. When pymetrozine was sprayed on a commercial tomato crop in a glasshouse in Switzerland for the control of aphids and whiteflies, it gave perfect control of both pest groups and allowed a complete parasitization of surviving pest specimens. Therefore pymetrozine can be considered as a unique case of combining both excellent pest control and outstanding selectivity.

19-028

NEW INSECTICIDE CHEMISTRY FOR CONTROL OF AUSTRALIAN PLAGUE LOCUST *CHORTOICETES TERMINIFERA* (WALKER) AND SPUR-THROATED LOCUST *AUSTRACRIS GUTTULOSA* (WALKER) IN AUSTRALIAN AGRICULTURE.R.M. Bull, G.H.S. Hooper¹

Rhone-Poulenc Rural (Australia) Pty Ltd, Brisbane, Australia - ¹ Australian Plague Locust Commission, Canberra, Australia.

A new phenyl pyrazole insecticide, Fipronil® was shown in field trials and a laboratory topical application assay to have high activity at very low doses against two of the major Australian locust species, *C. terminifera* and *A. guttulosa*.

Optimum field application rate was 7.5-10g active ingredient (AI) per hectare which achieved 80-100 percent mortality of both adult and juvenile stages in 1-3 days and was significantly ($P < 0.05$) better than the commercial standard, fenitrothion at 450g AI per hectare.

Serial introduction of fresh locusts into cages in treatment plots demonstrated good residual activity of Fipronil on rangeland herbage for up to 9 days after treatment while the intended commercial rate of Fipronil of 7.5-10g AI per hectare produced higher mortality and longer residual than fenitrothion.

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19-030

PYMETROZINE: A NOVEL APHICIDE WITH A NEW MODE OF ACTION

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Pymetrozine is the first representative of a new chemical class, the pyridine azomethines. The mode of action is different from all known mechanisms: an immediate feeding stop is followed later by death through starvation. Pymetrozine is active against aphids, whiteflies and hoppers in a large range of crops. Favourable toxicity and ecotoxicity profiles and its selectivity towards beneficial arthropods all contribute to an excellent fit into Integrated Pest Management programmes. Pymetrozine is highly effective in reducing the vectoring ability of virus-transmitting aphids.

The following aspects are covered in more detail:

- * Pymetrozine is not affected by cross resistance with established aphicides. This makes it a promising tool for resistance management.
- * Pymetrozine can be successfully applied through drip irrigation to control aphids in vegetables and in citrus. Pymetrozine is highly selective even with foliar application, however, application via the drip irrigation has several major advantages for the farmer. Workload is considerably reduced, and duration of control is increased. A longer duration of activity was observed with developing as opposed to fully developed plants.
- * Clear visual symptoms have been identified allowing to recognise the feeding stop before actual death of the pests.
- * The selectivity against bumble bees has been confirmed in a number of large scale trials. Pymetrozine applied to various crops in the glasshouse did not influence the activity of the bumble bees nor their pollination efficacy.

19-031

CONTROL OF CODLING MOTH (*CYDIA POMONELLA*) WITH SIRENE® CM, A NOVEL ATTRACT & KILL FORMULATIOND. Hofer¹, P. Charmillot², M. Angst¹

1) Ciba - Geigy AG., Crop Protection Division, CH - 4002 Basle, Switzerland; 2) Federal Agriculture Research Station, CH - 1260 Nyon, Switzerland

SIRENE® CM is a novel product for the control of codling moth (*Cydia pomonella*) in apple using the attract & kill concept to interrupt the life cycle at the adult moth stage.

The viscous slow release formulation based on a UV-absorber contains 6% of a contact insecticide and 0.16% of the pheromone codlemone as attractant. 150 or 300 g/ha of the formulation is applied in the form of 50 µl or 100 µl drops respectively. Application onto the leaves or branches is carried out by hand with a specially developed application system.

In 1995, registration trials were implemented in Switzerland after several years of successful testing SIRENE® CM.

SIRENE® fits very well into apple IPM programs due to its efficacy, selectivity and targeted application of low amounts of insecticide.

SIRENE®: Ciba - Geigy registered trade mark

19-033

MOLECULAR CLONING OF THE cDNA ENCODING THE PRO-PHENOLOXIDASE OF *HYPHANTRIA CUNEA* (LEPIDOPTERA: ARCTIIDAE)H.Y.Park, D.S.Park, S.S.Park, S.W.Shin, M.K.Kim, W.J.Lee¹, P.T.Brey¹

Insect Resources Laboratory, Korea Research Institute of Bioscience & Biotechnology, KIST, Taejeon, Korea -1 Unit of Ecology, Institut Pasteur, Paris, France

A cDNA clone encoding pro-phenoloxidase [pro-PO; zymogen of phenoloxidase (monophenol, L-dopa: oxydoreductase, EC 1.14.18.1)] was isolated from a Uni-ZAP XR library originated from the fall webworm, *Hyphantria cunea* larvae. This clone obtained with a 3.1kb insert that contained 5' initiation region and poly A signal.

The amino acid sequence was deduced from the respective cDNA. A hydrophobic NH₂-terminal sequence for a signal peptide is absent. The potential copper-binding sites shows high sequence similarity to POs especially *Bombyx mori* pro-PO P₁ and hemocyanins of arthropods. The overall cDNA sequence homology between fall webworm and other insect pro-POs ranged about 55-65%.

This clone was characterized by northern hybridization, southern hybridization and gene expression in insect cells.

19-032

PLANT MEDIATED EFFECT ON THE EFFICACY OF PYMETROZINE AGAINST APHIDS.

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Pymetrozine (CGA 215'944) is a selective insecticide against plant sucking pests. It acts as a feeding inhibitor with a subsequent starvation to death. Highest efficacy is observed after food intake and much less after topical contact. Hence, in order to obtain a good efficacy the compound should reach the feeding site of the insect. Fortunately, pymetrozine is taken up by plants and translocated within the vascular system, the phloem and xylem. Thus, the compound is distributed over the whole plant and occurs at the same time at the main feeding site of aphids, the phloem. In such systems, plants as intermediate are known to be a determining factor for a high activity and persistence. In the case of pymetrozine most of the plant parameters tested, such as physiological development stage, age, shoot penetration, metabolism rate and soil type had no influence on the efficacy. In contrast plant species dependent efficacy was observed: against *Myzus persicae* a 40 time higher activity has been found on tomato than on sugar beet plants. This may be a consequence of local distribution of pymetrozine within leaf tissues. In fact, one third of extractable compound was found in the intercellular washing fluids of tomato leaves as opposed to only 5% in sugar beet leaves. Thus, a considerable amount of penetrated pymetrozine is localized in the intercellular space of tomato plants, but not in sugar beet. However, this distribution pattern alone does not account for all differences in activity observed, specially in cases where the efficacy of pymetrozine on two plant species is similar against one aphid species and very different against a second aphid species, as seen on pea seedling and sugar beet plants against *Aphis craccivora* and *M. persicae*. Such variations in efficacy may be explained by plant species dependent feeding behaviour of the aphids.

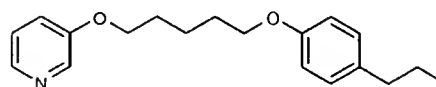
19-034

PRECOCIOUS METAMORPHOSIS-INDUCING ACTIVITY OF A NOVEL SERIES OF 3-PYRIDYL ETHERS

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We have recently found that a new series of 3-pyridyl ethers induce precocious metamorphosis in larvae of the silkworm, *Bombyx mori*. Both 2- and 4-pyridyl ethers were completely inactive, indicating that 3-pyridine moiety was essential for the activity. Octyl, dodecyl, and farnesyl 3-pyridyl ethers had no activity. Among the compounds tested so far, 5-(4-propylphenoxy)pentyl 3-pyridyl ether showed the highest activity. The activity fell off with increasing or decreasing length of the carbon chain between two oxygen atoms. Introduction of a methyl group at the 6 position of the pyridine ring completely eliminated the activity. Precocious metamorphosis induced by 3-pyridyl ethers was counteracted by a simultaneous application of a small amount of tebufenozide, an ecdysteroid agonist, suggesting that 3-pyridyl ethers caused ecdysteroid deficiency in the larval hemolymph to induce precocious metamorphosis.



19-035

SPINOSAD: AN OVERVIEW OF THE INSECTICIDAL ACTIVITY OF THIS NOVEL BIOLOGICALLY-DERIVED COMPOUND

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Spinosad belongs to a novel class of macrocyclic lactones produced by the soil actinomycete *Saccharopolyspora spinosa*, recently discovered by DowElanco. The compound is a complex of several natural metabolites, being spinosyns A and D, the two most active factors identified up to day.

Spinosad acts both as contact and stomach poison on the nervous system in a new manner, but the actual molecular mode of action is still unknown.

Furthermore, this new insect control agent has a very low mammalian toxicity.

In a variety of laboratory tests, the compound has been tested against 7 species of 5 orders, and the best results have been achieved against dipterans and lepidopterans.

19-037

[6-CHLORO-3-PYRIDYLMETHYL-³H]NEONICOTINOIDS: HIGH AFFINITY RADIOLIGANDS FOR THE NICOTINIC ACETYLCHOLINE RECEPTOR

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Neonicotinoids, acting at the nicotine binding site of the nicotinic acetylcholine receptor (nAChR), are one of the most important new classes of insecticides. Imidacloprid, the first commercial member of this new class, was synthesized in radioactive form with a specific activity of 25 Ci/mmol¹ and provided an outstanding radioligand that undergoes high affinity specific binding to the nAChR in house fly head membranes.² Since then several neonicotinoids have been prepared at high specific activities by methods applicable to neonicotinoids in general.³ These tritium-labelled compounds have helped greatly in mode of action research on the insect nAChR.⁴⁻⁷

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19-036

INTERNATIONAL STUDIES OF THE NEWEST ACRIDICIDE : THE FUTURE OF FIPRONIL

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Fipronil is an insecticide developed by Rhône-Poulenc Company. It belongs to a new family of phenyl-pyrazoles. Fipronil acts by blocking the GABA-regulated channels in the synaptic transmissions of the insect nervous system. It possesses a systemic action, being toxic both by ingestion and contact. Applied at very low rates (about 10 g of active ingredient per hectare), it causes the wholesale mortality in locust or grasshopper populations within a few days after treatment. The field tests of Fipronil were conducted in 1995 in Lake Baikal Region (East Siberia, Russia), to control temperate non-swarving grasshoppers. The community of grasshoppers exposed to treatments included 10 species, predominantly of Gomphocerinae subfamily. Age varied from first-instar hoppers to adults, the average grasshopper density was 28.8 individuals/m². Applied at the rate of 7.2 g a.i./ha, Fipronil caused 81-87% mortality of grasshoppers 24 h after treatment. Tests conducted in 1994-1995 in Mauritania by CIRAD-PRIFAS (France) established the rates of Fipronil efficient to control the Desert locust: 6.25 g/ha for total area treatments and 12.5 g/ha for barrier treatments. In these tests, the mortality of hoppers close to 100% was achieved 2-3 days after treatments.

Very high efficacy at extremely low dosages, good soil and foliar persistence, limited soil mobility, systemic action and formulation / application flexibility allow to consider the Fipronil as an outstanding acridicide, possibly a candidate to substitute the Dieldrin in locust and grasshopper control. It is virtually non-toxic for plants and mammals and low toxic for fish and birds. However, being highly toxic for many arthropods, further investigations are needed to evaluate the effects of the Fipronil on non-target arthropod fauna.

19-038

TOXICITY OF LARVIN® BRAND THIODICARB AND THREE OTHER INSECTICIDES TO SUSCEPTIBLE AND RESISTANT STRAINS OF TOBACCO BUDWORM *HELIOTHIS VIRESCENS* COLLECTED FROM DIFFERENT SITES IN THE USA. COTTON BELT

H.M. Ayad, R. G. Blenk, S. P. Schmidt and J. B. van Kretschmar.
Rhône-Poulenc Ag Co., Research Triangle Park, North Carolina, USA.

The results we accumulated over the last six years illustrated the consistence performance of Larvin® against susceptible and resistant populations of tobacco budworm collected from different sites in the cotton belt. These data are in agreement with our laboratory data that showed subjecting tobacco budworm larvae or eggs to the selection pressure of Larvin did not cause significant increase in the level of resistance to this compound. Another reason for Larvin consistence performance is our findings that small difference exists between its toxicity to the eggs collected from susceptible and resistant populations.

A careful examination of resistance ratios indicated that larvae collected from sites in the mid-south and south-west possessed high level of resistance to synthetic pyrethroid insecticides. Our biochemical studies show no significant difference between the penetration rate of Larvin in susceptible and resistant larvae. Also, Larvin was almost equitoxic to the acetyl cholinesterase enzymes separated from the heads of susceptible and resistant larvae.

19-039

SPATIAL DISTRIBUTION OF TEFLUBENZURON RESISTANCE BY PEAR PSYLLA IN WESTERN SWITZERLAND

L. Schaub and B. Bloesch, RAC Changins, Nyon, Switzerland

In recent years, pear producers in the region of Lake Léman observed an efficacy reduction of teflubenzuron against pear psylla (*Cacopsylla pyri* L.). Thus, in 1994 and 1995, we conducted experiments in the laboratory to confirm the efficacy reduction and to study the spatial distribution of the resistance in western Switzerland.

After application of a registered dose of teflubenzuron, all eight samples from orchards of the region of Lake Léman showed low mortality rates whereas the nine samples from the Valais showed normally high mortality rates. The estimation of the dose-mortality response of psylla from one orchard of each region confirmed these observations.

We conclude that the psylla of the Lake Léman region were resistant to teflubenzuron. Also, psylla from an organic orchard without any teflubenzuron treatment were resistant. This indicates that movement played an important role in psylla resistance development.

19-041

INHIBITION OF PYRETHROID RESISTANCE ESTERASES BY PIPERONYL BUTOXIDE IN AUSTRALIAN *HELICOVERPA ARMIGERA* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE).

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The cotton bollworm *Helicoverpa armigera* (Hübner) is a serious pest of cotton and other summer crops in Australia, where it has a long history of insecticide resistance, (DDT, pyrethroids, carbamates, organophosphates and endosulfan). Chemical insecticides are currently essential for the control of *H. armigera* on cotton and are likely to remain an important component of control strategies for the foreseeable future.

Despite some pyrethroid synergism by piperonyl butoxide (Pbo), esterase mediated detoxification of pyrethroids has been found to be a major mechanism of resistance in *H. armigera*. This is due to the overproduction of esterase isoenzymes which sequester and hydrolyse pyrethroids.

Data presented in this paper shows that piperonyl butoxide is an effective inhibitor of esterase mediated hydrolysis of 1-naphthyl acetate in *H. armigera*. Up to 50% of enzyme activity, in the pyrethroid resistance related esterase, could be inhibited by piperonyl butoxide. Concentrations of Pbo which produced this effect, are similar to the internal concentrations of Pbo which synergise pyrethroids against *H. armigera*. We suggest that this esterase inhibition, is at least partially responsible for pyrethroid synergism by Pbo in Australian *H. armigera*.

19-040

DEVELOPMENT OF RESISTANCE TO MULTIPLE CLASSES OF INSECTICIDES IN *HELIOTHIS VIRESCENS* (F.) (LEPIDOPTERA: NOCTUIDAE) POPULATIONS IN THE U.S. MID-SOUTH

G. W. Elzen

USDA, ARS, Southern Insect Management Laboratory, Stoneville, MS USA

The tobacco budworm, *Heliothis virescens* (F.), an important pest of cotton in the United States, has developed resistance to nearly every class of insecticide used in cotton. Effective management of resistance depends in part upon a knowledge of insecticide resistance levels. Several different bioassays have been used during the past decade to characterize resistance in this pest. This paper summarizes findings of these studies and presents results of recent evaluations of resistance levels and cross-resistance patterns.

Resistance to pyrethroid insecticides was first detected in the 1985 and 1986 cotton growing seasons in West Texas, Louisiana, and Mississippi. Increased tolerance to carbamates and the cyclodiene endosulfan was first detected in 1989 and 1990, respectively, in Mississippi. Resistance to carbamates and organophosphorus insecticides was confirmed in Louisiana and Mississippi in 1990. Resistance to endosulfan was confirmed in a tobacco budworm strain collected in Mississippi in 1992. Through 1995, resistance levels remained higher to pyrethroids than to carbamates. The organophosphorus insecticides, with low levels of resistance, remained the more effective materials. No cross-resistance was found between different classes of insecticides with the exception of carbamates and organophosphorus insecticides. Currently, the number of alternative insecticides is limited.

19-042

ESTABLISHING INSECTICIDE SUSCEPTIBILITY BASE-LINES FOR *HELIOTHINE* CATERPILLARS, USING IRAC BIOASSAY METHOD NO 7

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In order to monitor resistance levels in arthropod pests, it is important to establish reliable dose-responses and LD₅₀'s in known susceptible strains, against which field-collected populations can be compared and resistance factors or ratios calculated. This IRAC-funded study has generated base-line dose responses in four strains of *Heliothine* caterpillars against seven compounds representing the major product chemistries used for control of cotton bollworms. Discriminating doses based on these data will be validated in field studies in 1996/97.

IRAC Method No 7 was used throughout, as this is being promoted as a convenient and versatile bioassay procedure for leaf-feeding pests. Some strengths and weaknesses of the method are presented. A full report of the study is available from the authors.

19-043

RAPID SELECTION OF MALATHION RESISTANCE IN THE CAT FLEA *CTENOCEPHALIDES FELIS* (BOUCHE) (SIPHONAPTERA: PULICIDAE)
E. W. Moyses, A. Buchy, Ciba-Geigy, Animal Health, Basel, Switzerland.

Cat fleas were bred in the laboratory with the aid of an artificial feeding system. Susceptibility was measured by 24 hour exposure of unfed adults to insecticide deposits on filter paper. The LC_{50} of a field strain from Greensboro, NC, USA was 93 mg malathion/ m^2 . The corresponding "baseline" strain value was 63 mg. The Resistance Ratio (RR) was therefore 1.5. At the LC_{90} level the values were 400 and 140, respectively (RR = 2.8). A small fraction of the field strain population (3%) was not killed at 800 mg malathion/ m^2 .

Malathion selection pressure was applied, using the same methodology, first with 400 mg/ m^2 then in further generations with: 800 mg/ m^2 (F_1 to F_{11}) and 1,600 mg/ m^2 (F_{12} to F_{17}). Malathion resistance developed rapidly. The mortalities due to the selection process were: generation F_0 - 91%; F_1 - 45%; F_2 to F_{11} - average 17%; F_{12} to F_{17} - average 11%. Bioassays were made in F_2 , F_{14} and F_{16} with various concentrations of malathion. At the highest concentration tested (12,800 mg/ m^2) the highest mortality was 30% in F_{16} (RR = 260). Generations F_{18} and F_{19} were selected with 12,800 mg malathion/ m^2 . The resultant mortalities were 23% and 42%, respectively.

Higher mortalities could be obtained with topical application of very high dosages. A mortality of 95% (after 48 hours) was reached in F_{19} with a dosage of 20,000 ng malathion/insect. The corresponding value for the baseline strain was 29 ng. The RR at LD_{95} was therefore 690. This is, as far as we know, by far the highest resistance ratio recorded in the cat flea. The RR for malaoxon, in a similar comparison was only 18. These results are discussed.

19-045

ASPECTS OF INSECTICIDE RESISTANCE AND REPRODUCTIVE BIOLOGY OF *APHIS GOSSYPYII* GLOVER ON SEED POTATOES

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Istituto Sperimentale per le Colture Industriali, Bologna, Italy

For several years the aphid population patterns and the incidence of the main potato virus diseases have been monitored in Italian potato-growing areas. Starting from 1992, an increase in *Aphis gossypii*, as compared to *Myzus persicae*, *Macrosiphum euphorbiae* and *Aphis fabae*, was observed on seed potato fields in the north of Italy.

Laboratory tests revealed that *A. gossypii* was insensitive to the pesticide Pirimicarb, even at the maximum dose tested (2400 $\mu\text{g.ml}^{-1}$). Moreover, in the presence of Pirimicarb the reproductive capacity increased up to 28% as compared to the control and the new-born aphids began to appear 2 days earlier in the treated group. *M. persicae*, on the other hand, was controlled at a lower dose (300 $\mu\text{g.ml}^{-1}$). Lambda-Cyhalothrin (Karate) and Imidacloprid (Confidor) were very effective against both aphids.

19-044

GEOGRAPHIC VARIATION IN TOLERANCE OF THE RICE YELLOW STEM BORER (LEPIDOPTERA: PYRALIDAE) TO *BACILLUS THURINGIENSIS* TOXINS

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³Department of Entomology, North Carolina State University, USA.

The yellow stem borer (YSB), *Scirpophaga incertulas*, is a major pest of rice in Asia and one of the principal targets of genetic engineering of rice with toxins from *Bacillus thuringiensis* (Bt). We examined geographic variation in tolerance of YSB to three Bt toxins, CryIA(c), CryIC, and CryIIA, that are highly toxic to YSB in the Philippines. Among populations collected on Luzon Island, Philippines, LC_{50} values did not differ among collection sites within towns or among towns within provinces. However, we detected small but significant differences in LC_{50} s for all three toxins among populations from three provinces.

YSB from China and Vietnam were more tolerant of all three toxins than were YSB from the Philippines. The LC_{50} of CryIC and CryIIA was up to 200-fold and 70-fold higher, respectively, in YSB from China than from the Philippines. Bt plants genetically engineered with CryIA(c), CryIC, or CryIIA should perform similarly well across the area of the Philippines that we studied. However, plants with CryIC and CryIIA may not perform as well in China, unless levels of toxin production are high enough to account for the possible higher tolerance of local YSB populations.

19-046

Acetylcholinesterase and P450 genes and insecticide resistance in *Heliothis virescens*.

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Heliothis virescens is a pest of cotton and tobacco in South Carolina. Resistance to methyl parathion was detected in 1978. Most cotton growers have changed to synthetic pyrethroid insecticides. After 18 years of application of synthetic pyrethroids, resistance to this class has been detected in South Carolina.

A portion of the acetylcholinesterase gene of *H. virescens* was amplified by the polymerase chain reaction (PCR) using degenerate primers designed from known sequences of other insects and employed in a nested procedure with RNA as template. The resistant form of this enzyme confers 20-fold resistance to methyl parathion. Also, PCR primers were designed to amplify the CYP9 gene of *H. virescens* from genomic DNA as template and a putative mutation in this gene will be described. Progress in linkage mapping of resistance genes and marker genes in this species will be reported.

19-047

ANTIFEEDANT-EFFECT, BIOLOGICAL EFFICACY AND HIGH AFFINITY BINDING OF IMIDACLOPRID TO ACETYLCHOLINE RECEPTORS IN TOBACCO ASSOCIATED *MYZUS PERSICAE* (SULZER) AND *MYZUS NICOTIANAE* BLACKMAN (HOMOPTERA: APHIDIDAE)

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Myzus persicae (Sulzer) and *Myzus nicotianae* Blackman are two closely related homopteran pests and resistance to carbamates, organophosphates, and pyrethroids due to elevated levels of carboxylesterases and insensitive acetylcholinesterase is now widespread in both species.

Imidacloprid, a chloronicotinyl insecticide acting agonistically on nicotinic acetylcholine receptors, controls resistant aphid strains of both species due to its lack of any bonds susceptible to degradation by esterases. Nevertheless earlier studies as well as this study showed that in particular tobacco associated forms of *M. persicae* and *M. nicotianae* seemed to be somewhat less susceptible (5- to 10-fold) to this insecticide than susceptible reference strains.

Furthermore *M. nicotianae* seems to be more tolerant to antifeedant effects evoked by sublethal concentrations of imidacloprid relative to a susceptible reference strain. Antifeedant effects were bioassayed using a floating-leaf choice test and by counting excreted honeydew droplets.

Receptor binding studies with [³H]-imidacloprid and [³H]-nicotine in homogenates of *M. nicotianae* and *M. persicae* revealed no differences between susceptible and tolerant strains. Thus indicating that target site insensitivity is apparently not the mechanism for a 5- to 10-fold imidacloprid tolerance in *Myzus nicotianae*.

19-049

RESISTANCE OF TETRANYCHUS URTICAE KOCH. (TETRANYCHIDAE) TO BISCLOFENTEZIN

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Investigation were carried out in the "Dedinje" glasshouse for flower growing (carnation, rose, etc.) in Belgrade. Over the 1984-1989 period the population of *Tetranychus urticae* was high and it considerably endangered flower production in almost all glasshouses in Serbia. Over the above-mentioned period two treatments per year were applied (Bisclofentezin combined with Cihexatin), providing successful flower protection against acarinae. Bisclofentezin was therefore applied until 1992. However, since 1988 the effect of Bisclofentezin has been observed to be considerably lower, especially in the last few years. The objective of this paper was to determine the resistance degree of *T. urticae* after Bisclofentezin has been applied for ten years.

The results obtained in this study show high degree of resistance in the studied population after 10 years of permanent treatments with Bisclofentezin. It is necessary to use some other acaricides instead of Bisclofentezin in the glasshouses where Bisclofentezin has been permanently applied for many years.

19-048

The *in vivo* mechanisms of housefly resistance to permethrin Yoji Takada, Takao Ishiwatari, Makoto Hatakoshi and Masachika Hirano

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In order to know the mechanisms of resistance to pyrethroids, the houseflies (*Musca domestica*) collected at Akagi, Gunma prefecture (Akagi colony) in Japan were used in this study. Permethrin selected Akagi PP15 strain showed the high resistance to pyrethroids. An increased metabolism by microsomal cytochrome P-450 dependent monooxygenase (mfo) system was responsible, but esterase was not related to the resistance in the study using synergists. And the *in vivo* metabolism of permethrin was also studied using ¹⁴C labeled compound. But the difference of metabolic fate of permethrin between Akagi PP15 strain and insecticide susceptible strain was not observed up to two hours after topical application of the compound. The influence of penetration through cuticle was negligible by the study using ¹⁴C labeled compound. It was also clarified that this Akagi PP15 strain showed the low sensitivity to pyrethroids by the electrophysiological study *in vivo*. Furthermore, it was clear that the locus of the recessive resistance gene on the third chromosome was allelic to *kdr* gene already known. In conclusion, the main mechanisms of Akagi PP15 strain were the low sensitivity of the nervous system to pyrethroids and the increased metabolism by mfo.

19-050

TESTS FOR PYRETHROID RESISTANCE ON ANOPHELES POPULATIONS WHICH HAVE LONG BEEN EXPOSED TO IMPREGNATED BEDNETS

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The encouraging results from the use of pyrethroid impregnated bednets against malaria vectors is leading to rapid expansion of use of such nets. It is important to assess whether there is therefore an imminent threat of emergence of pyrethroid resistance.

Observation of the time for knockdown of the first, median and last mosquito when a sample is made to walk on an impregnated net has been found to be a sensitive means of distinguishing pyrethroid resistant and susceptible anophelines. This method, has been applied, together with conventional observations of % mortality after 1 hour's exposure to W.H.O. impregnated papers. Observations were made on malaria vectors in two areas where impregnated nets have been extensively and continuously used since 1987 - in Sichuan, China, and in the village of Mng'aza in north east Tanzania. In each case comparison was made with the same species in areas where there has been no known use of pyrethroids. No evidence was found for resistance.

Time for knockdown by pyrethroids is reduced in heat stressed and older mosquitoes and precautions to avoid misleading results from these causes are required.

19-051

GENETIC STUDY OF DELTAMETHRIN RESISTANCE IN *ANOPHELES STEPHENSI* LISTON, A MALARIA MOSQUITO.

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An.stephensi is one of the important vectors of malaria in Indian sub continent. It is a member of the sub-genus *Cellia* and series *Neo cellia*. Mosquitoes have developed resistance to various types of insecticides including organochlorides and organophosphates. Deltamethrin has been extensively used in India because of its biodegradability and low mammalian toxicity to control *Anopheles* vectors including *An.stephensi*.

A diagnostic dosage of 0.004 ppm of Deltamethrin was used to separate the laboratory produced homozygous resistant, F_1 hybrids, and susceptible strains. The progeny of the backcrosses and F_2 generations were also treated with the above dosage. The data on resistance, susceptibility and time-mortality relationship shows that Deltamethrin resistance was incompletely dominant and autosomal. There is a remarkable variation in respect of the sex ratio, fecundity and egg hatchability between the resistant and susceptible strains.

Total as well as soluble protein content and general esterase activity in resistant strains has been found increased. The same was also compared using other insecticides such as malathion, Parathion and Fenthion, which have been studied in our laboratory.

19-053

A MECHANISM OF PERMETHRIN RESISTANCE IN THE SOUTHERN HOUSE MOSQUITO, *CULEX QUINQUEFASCIATUS* SAY, FROM SAUDI ARABIA

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Culex quinquefasciatus larvae from Saudi Arabia (JPal-per) showed high levels of resistance (2,500-fold) to permethrin. Cross resistance of this strain to other pyrethroid insecticides and DDT was also observed. The resistance ratio for different pyrethroids with and without α -cyano groups in the alcohol moiety, as compared to the susceptible(S) strain varied significantly from 5.6-fold to 4,200-fold. The pyrethroids lacking an α -cyano group but having a phenoxybenzyl group in their chemical structure showed the highest resistance ratio.

A major contribution of P450 oxidases in the degradation of pyrethroids in JPal-per strain was indicated by large differences in the synergistic effects of oxidase inhibitors such as PBO and TCPE (2-propynyl 2,3,6-trichlorophenyl ether) on permethrin toxicity between the JPal-per and S strains. The amounts of P450 and b5 were also observed to be 2.7 and 2.9-fold higher in the JPal-per strain as compared to the S strain.

Previous studies have shown that *in vitro* metabolism tests can't be carried out perfectly due to an unknown inhibitor in the larval body. In our study we found the inhibitor in the contents of the larval gut and this made it possible to measure the P450 monooxygenase activity by removing the contents from gut. The microsomal fraction of JPal-per strain showed NADPH dependent high activity of permethrin degradation as compared to the S strain. Furthermore, the activity of P450 monooxygenase was greatly inhibited by oxidase inhibitors, TCPE and PBO. Therefore it was concluded that cytochrome P450 monooxygenase plays a major role as a mechanism of permethrin resistance in JPal-per strain.

19-052

RESISTANCE MECHANISMS IN PYRACLOFOS-RESISTANT HOUSEFLIES

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Houseflies collected from Yumenoshima, a dumping island in Tokyo Bay, were selected with an organophosphorus insecticide, pyraclofos ((R,S)-[O-1-(4-chlorophenyl)pyrazol-4-yl O-ethyl S-n-propyl phosphorothioate], Boltage®) for 13 generations to obtain a strain resistant to the chemical. The selected housefly (YBOL) strain showed a high resistance ratio of about 650 times that of the susceptible reference (SRS) strain. Acetylcholinesterases (AChE) of the YBOL strain were 70 times less sensitive to inhibition by pyraclofos than the SRS strain. Piperonyl butoxide (PB), inhibitor of P450 monooxygenase, increased toxicity of pyraclofos in the SRS strain, but it decreased pyraclofos toxicity in the YBOL strain.

There were no differences in penetration rate in both strains. The metabolic rate of pyraclofos *in vivo* was almost the same in both strains. Major metabolites were, however, greatly different between the resistant and susceptible strains. The YBOL housefly mainly cleaved the P-O-aryl bond and made 1-(4-chlorophenyl)pyrazol-4-yl glucose and 1-(4-chlorophenyl)pyrazol-4-yl sulphate conjugates whereas the SRS housefly primarily cleaved P-S-n-propyl bond and produced 1-(4-chlorophenyl) pyrazol-4-yl O-ethyl hydrogen phosphorothioate. The enzyme concerned with cleavage of P-O-aryl bond is thought to play an important role in the mechanism of pyraclofos resistance.

19-054

MOLECULAR EVOLUTION OF E4 AND FE4 B CARBOXYLESTERASE GENES AS INDICATED BY SEQUENCE VARIATION IN *MYZUS ANTIRRHINII* AND *MYZUS CERTUS* (HOMOPTERA: APHIDIDAE)

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Identification of the primary E4 and FE4 carboxylesterase loci in *Myzus persicae* is made difficult by the multiple and varied chromosomal sites occupied by the amplified genes in different biological clones of this species. Therefore, we complemented our work on *M. persicae* by investigating the molecular evolution of these genes in closely-related members of the *M. persicae* species group that do not display insecticide resistance, namely *M. antirrhinii* (a permanently parthenogenetic species) and *M. certus* (a species that can be holocyclic). The sequences of introns 6 and 7 differentiate the E4 and FE4 genes of *M. persicae*. Using synthetic oligonucleotide primers complementary to sequences conserved between E4 and FE4 exons flanking introns 6 and 7, we have successfully amplified by PCR homologous gene fragments from both *M. antirrhinii* and *M. certus*. Comparative sequence analysis of these amplified fragments demonstrated for the first time that both E4 and FE4 genes could occur together naturally in individual biological clones of the *M. persicae* species group. Southern hybridization and quantitative dot-blot experiments showed that each of these two species has 2 to 5 copies of E4/FE4 genes. Recombinant DNA clones containing 3' flanking regions of *M. antirrhinii* E4/FE4 genes were isolated from a genomic library made with bacteriophage lambda DASH II, and comparative analysis of the flanking sequences is being used to investigate the mechanisms of gene amplification and dispersal to different loci.

19-055

MONITORING FOR THE RESISTENCE OF DIAMONDBACK MOTH (*PLUTELLA MACULIPALPA*) IN THE FIELD

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By the diagnostic tests, the resistance of *Plutella xylostella* (Lepidoptera: Plutellidae) in Wuhan areas has been tested to Cascade (flufenoxuron), Sumicidin (fenvalerate), Lannate (methomyl) and Phosmet with the LC_{50} of sensitive strain as the discriminating doses and employing the leaf impregnation method. The results revealed that the resistance of *P. xylostella* to Cascade had risen by 12-14-fold during the period of 1991-1994, 136-263-fold to Sumicidin, and 193-233-fold to Phosmet, but no significant rising has been found to Lannate. It is suggested that, in Wuhan areas, the employment of pyrethroid and organophosphate pesticides should be forbidden, monitoring the application of insect growth regulators as Cascade and Atabron (chlorfluazuron), and the alternative use of Lannate and other insecticides has been recommended.

19-057

DIFFERENT MECHANISMS IN THE *Culex pipiens* MOSQUITO LARVAE (DIPTERA: CULICIDAE) RESULT IN RESISTANCE TO *Bacillus sphaericus*

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Recently field *Culex pipiens pipiens* mosquitoes collected after a control failure with Spherimos, substitute of conventional insecticides, in southern France developed a very high resistance (> 10,000 fold) to *Bacillus sphaericus* (Bsp) crystal toxins after a few generations of laboratory selection. We show that this resistance is (a) encoded by a single major recessive gene localized on linkage group I at 22.1 units of recombination from the sex factor, (b) not associated with the presence of serine proteins like the over-produced esterases involved in organophosphorous resistance and (c) not associated with any loss of binding affinity between brush border membrane fractions (BBMFs) and the Bsp radiolabeled toxin, in contrast to the high Bsp resistance developed after laboratory selection in Californian *C. p. quinquefasciatus*.

It thus appears that at least two different mechanisms may confer high levels of resistance to *B. sphaericus* crystal toxin in mosquitoes of the *Culex pipiens* complex. These results are discussed in relation to mosquito control strategies and resistance mechanisms.

19-056

DEVELOPMENT OF INSECTICIDE RESISTANCE IN AN ESTABLISHED CELL LINE OF MEDITERRANEAN FRUIT FLY (*CERATITIS CAPITATA* Wied.) (DIPTERA: TEPHRITIDAE).

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Insect species have shown a generalized capability to develop mechanisms for surviving to biocides. This capacity is not the same in all species. Populations of Med Fruit Fly artificially selected in the lab or exposed to chemical treatments in the field did not develop resistance to insecticides or they did only slightly. In this work we tested the established cell line CCE/CC128 of *Ceratitis capitata* for the ability to develop resistance to insecticides. The cells were challenged for several cycles with 90 µg/ml of Malathion, an organophosphorous insecticide largely used in the control of this species in the field. After each cycle of treatment the cells were counted and the inhibition of cell proliferation was calculated. The percentage of inhibition was initially high, thereafter a progressive reduction was observed. After a period of three months, the Malathion selected population was not susceptible any more to the dose of 90 µg/ml of the biocide. The kinetics of cell proliferation at different doses of Malathion confirmed the selection of a cell population resistant to this insecticide. With the aim of understanding the biochemical basis of this resistance, we started to examine the proteins for which we have *a priori* expectations of involvement with resistance. The main detoxifying enzymes such as esterases and glutathione S-transferase are actually under investigation. Afterwards, the structure and expression of involved genes will be characterized.

Similar experiments with different insecticides are in progress.

19-058

CLONING AND CHARACTERIZATION OF THE CARBOXYLESTERASE GENE FROM THE BROWN PLANTHOPPER, *Nilaparvata lugens* (Stål)

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The brown planthopper (BPH), *Nilaparvata lugens* (Stål) is one of the most serious pest insects of rice plant in Asian countries and it has developed resistant to some insecticides.

Carboxylesterases (CEs) are reported to be involved in resistance to certain OPs and pyrethroids in this species. To study the molecular genetic basis of resistance to OPs, we cloned and characterized the DNA fragments of the CE gene in BPH.

Several molecular forms of CEs are observed in this insect. We prepared polyclonal antisera to the three major forms of CEs by injection of respective forms into mice. These antisera crossreacted to the other forms of CE in BPH. The BPH lambda gt11 cDNA library was screened using antiserum against the most active form of CE.

More than ten positive clones were isolated and partial sequence analysis showed that all these clones were almost identical to each other. These sequences are closely related to CE of the small brown planthopper. However, the DNA sequences of CE from the BPH did not have high identity with those of the other known insect CEs.

19-059

MOLECULAR CHARACTERIZATION OF KNOCK-DOWN RESISTANCE (*Kdr*) TO PYRETHROID INSECTICIDES IN THE GERMAN COCKROACH

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Knock-down resistance (*kdr*) to DDT and pyrethroid insecticides was reported in several important insect pest species, including the German cockroach. Genetic linkage analysis has shown that *kdr* resistance in the German cockroach, house fly, and tobacco budworm is linked to the *para*-homologous sodium channel gene, suggesting that a mutation(s) in the *para*-homologous sodium channel gene results in *kdr* resistance. *kdr* resistant strains are cross-resistant to several site 2 sodium channel neurotoxins.

para-homologous sodium channel genes of a pyrethroid susceptible strain and a *kdr*-type strain of the German cockroach have been cloned and sequenced. Comparison of amino acid sequences deduced from these cDNA clones revealed several differences between the two *para*-homologous sodium channel genes. In this poster, the amino acid sequence comparison results will be presented. The possible involvement of these amino acid changes in the *kdr* mechanism will be discussed.

19-061

INSECTICIDE RESISTANCE GENES IN *Culex pipiens* FROM SARDINIAE. Pili¹, G. Addis¹, M. Raymond², A. Marchi¹

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Populations of *Culex pipiens* from different parts of Sardinia were analyzed for susceptibility to organophosphorous insecticides (OP) and for the presence of overproduced nonspecific esterases and of insensitive acetylcholinesterase, two major mechanisms of OP and carbamate resistance in this species. Resistance was found to be positively correlated with the frequency of insensitive *Ache* genes. Several overproduced esterases of A and B type were found in the resistant populations. However their frequency was always too low to be primarily responsible of OP resistance, except for the associated esterases A4 and B4 which high frequency was correlated with high resistance levels. Esterase A1 and the associated esterases A2 and B2 were concentrated in the Southern part of the island and always linked to high frequency of A4-B4. Monitoring of populations under routine insecticide treatment was conducted over a five year period to detect changes in frequency of resistance genes. Gene amplification responsible of B esterase overproduction was confirmed by RFLP analysis using a 1.3 Kb B1 probe. Restriction patterns of the amplified B regions were identical to those of other Mediterranean populations and of reference strains, sustaining the hypothesis of a unique origin of the amplification event.

19-060

INSECTICIDE RESISTANCE IN ITALIAN *CULEX PIPPIENS* FIELD POPULATIONSV. Di Pardo¹, C. Severini¹, F. Silvestrini¹, M. Marinucci¹, G. Pierdominici¹, R. Romi¹, M. Raymond².

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The most widespread mechanisms of organophosphate resistance (OP) in *Culex pipiens* L. mosquitoes are overproduction of detoxifying nonspecific esterases and selection for insensitive acetylcholinesterase (AChE). In *Cx. pipiens*, the nonspecific esterases are encoded by two closely linked loci, named A and B. Overproduction of the B esterases is due to gene amplification, resulting in the B1, B2, B4 and B5 alleles, which generally are in linkage disequilibrium with specific A alleles. In Italy, previous studies on OP resistance in *Cx. pipiens* were carried out in only few areas.

A comprehensive geographical survey in Italian *Cx. pipiens* field populations was undertaken in order to evaluate the current mechanisms of OP resistance. 11 samples from 8 Italian regions have been studied; OP resistance was determined using WHO bioassays test procedures. AChE assay was carried out using propoxur as inhibitor; the different highly active esterases in each mosquito were detected using starch gel electrophoresis and DNA Restriction Fragment Length Polymorphism analysis. We found that the samples collected in regularly treated areas show high correlation between OP resistance and overproduced esterases patterns; in some of these areas OP resistance was determined also by the presence of insensitive AChE.

19-062

MONITORING AND DETECTION OF INSECTICIDE RESISTANCE IN *APHIS FABAE* AND *MYZUS NICOTIANAE* (HOMOPTERA: APHIDIDAE) IN GREECE.

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During 1993-95, fourteen populations of *Aphis fabae* Scopoli and thirty four populations of *Myzus nicotianae* Blackman were collected from sugarbeet and tobacco fields, respectively, in Northern Greece. Laboratory bioassays were conducted to evaluate the LC₅₀s for some of the insecticides used by farmers such as pirimicarb, methamidophos, fenvalerate and carbosulfan, and the recently used triazamate and imidacloprid. Resistance was determined using the FAO Dip Test method, with mortality assesment after 24 hours. Results from all bioassays suggested that *Aphis fabae* had no detectable insecticide resistance, but the probit analysis indicated that some populations were heterogeneous with shallow regression lines and high variability, suggesting that rapid development of homozygous resistance will occur, if selection pressure is continued.

Comparisons made among populations of *Myzus nicotianae* from different regions showed differences in the susceptibility to insecticides. Pirimicarb resistance was estimated from 2.5 to 939-fold, with LC₅₀ for the most susceptible field strain 30.04 (95%CL 19-47) ppm (AI). Tamaron resistance was up to 18.5-fold. LC₅₀ for the most susceptible strain was 40.16 (23-69) ppm. Triazamate has been used very little in the commercial fields. Despite that, resistance was detected from 5 up to 20-fold, probably because cross resistance has been occurred. LC₅₀ for the most susceptible population was 13.51 (8-23) ppm. No significant differences were found among populations for fenvalerate and imidacloprid. The pooled LC₅₀s were 4.84 (2.3-10.1) and 9.26 (4.17-15.8) ppm (AI), respectively.

19-063

SUSCEPTIBILITY OF VECTORS OF LEISHMANIASES IN VENEZUELA TO SELECTED INSECTICIDES

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Cutaneous and visceral leishmaniasis are endemic in Venezuela, being *Lutzomyia longipalpis* and *L. ovallesi*, respectively, recognized as vectors of these diseases in the country. Both species enter the houses, therefore a control programme based on the use of insecticides, indoor spraying or impregnated curtains, might be applicable. In order to determine the susceptibility to different insecticides, a laboratory strain of *L. longipalpis* and a field strain of *L. ovallesi* were challenged to organochloride (DDT 2%), organophosphate (Malathion 2%, fenitrothion 1% and pirimiphos methyl 1%), carbamate (prothion 0.01%) and pyrethroid (deltamethrin 0.06%, lambda-cyhalothrin 0.06% and permethrin 0.2%). The results show that *L. longipalpis* is susceptible to organophosphate, carbamate and pyrethroid insecticides, being the lethal time (LT₉₅) less than 60 minutes (27-47 min), whereas for the DDT the LT₉₅ was up to 60 min. However, a field population needs to be tested.

Preliminary data observed for *L. ovallesi* indicated a higher susceptibility to pyrethroid, deltamethrin and lambda-cyhalothrin insecticides. The LT₉₅ was 15 and 8 minutes, respectively.

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19-065

THE STRUCTURE-ACTIVITY RELATIONSHIP OF PYRETHROIDS AGAINST PERMETHRIN-RESISTANT AND SUSCEPTIBLE STRAINS OF ANOPHELES STEPHENSI LARVAE AND ROLE OF MIXED-FUNCTION OXIDASES IN RESISTANCE PROFILE

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Structure-activity relationship of pyrethroids (permethrin, deltamethrin, lambda-cyhalothrin, cypermethrin, cyfluthrin) against larvae of different geographical strains of *An. stephensi* revealed that α -cyano group of pyrethroids with dibromo substitutions are more toxic than the other classes to sensitive strains. The ranking order in toxicity of pyrethroids was different in resistant strains. Evidence of enhanced monooxygenase activity and the synergism shown by piperonyl butoxide suggests that each resistant strain possesses a monooxygenase-mediated resistance mechanism responsible, at least in part, for pyrethroid resistance.

19-064

REDUCED ACETYLCHOLINESTERASE SENSITIVITY IN DIAMONDBACK MOTH POPULATIONS IN SOUTH CHINA

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The diamondback moth, *Plutella xylostella* (L.) is an important pest of cruciferous vegetables in South China. In different regions the field populations of this moth have developed different levels of resistance to insecticides used. In recent years, farmers have expanded the cultivated area of vegetables, consequently there is a great need for developing some biochemical methods to detect and monitor the resistance in individual insect. It has been widely recognized and documented that in this moth acetylcholinesterase (AChE) insensitivity is the important mechanism of resistance. A method of dot-blot on nitrocellulose membrane for identification of insensitive AChE in single insect has created the opportunity for early detection and subsequent monitoring of resistance in field populations. The percentages of inhibition of AChE activity in resistant strain (GBR) and field population (DG) were 50.97% and 43.96% respectively in the presence of prothion, and were 43.76% and 35.87% respectively in the presence of paraoxon. The results showed that GBR and DG have higher degrees of AChE insensitivity to organophosphorus and carbamate than the susceptible strain.

19-066

HIGH RESISTANCE IN NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER TO LUFENURON, A CHITIN SYNTHESIS INHIBITOR INSECTICIDE

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Chitin synthesis inhibitors are potent insecticides against certain insects, including *Drosophila melanogaster*. The benzoylphenyl urea compound lufenuron was tested against both laboratory and natural population strains of this insect. Lufenuron exposure results in lethality in all preadult stages of development. When fed to adult females, lufenuron sterilizes the females due to embryonic lethality of the oviposited eggs. Laboratory strains were highly susceptible to this compound, while natural population strains from two widely separated locations in the U.S. exhibited considerable (as much as 100-fold) resistance. The resistance is being genetically mapped and compared with resistance of these strains to several other insecticides. Since *D. melanogaster* is not under direct insecticide selection pressure, these results suggest that strong selection for insecticide resistance can occur in insects indirectly affected by an insecticide.

19-067

INSECTICIDE RESISTANCE IN DIAMONDBACK MOTH *PLUTELLA XYLOSTELLA* L. IN INDIA : DISCRIMINATING DOSE STUDIES

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Discriminating doses for carbosulfan, cartap hydrochloride, fenvalerate, monocrotophos and quinalphos were fixed for different methods of bioassay viz., vial, leaf dip, larval dip, and spray tower. This involves : 1. Culturing the populations collected from fields less exposed to insecticide application for 25 generations without exposure to any insecticides, 2. Working out log-dose-probit-mortality lines (LDPM) for the above insecticides by vial, larval dip, spray tower and leaf disc bioassay methods using, third instar (1.83 ± 0.28 mg) larvae, 3. Extrapolation of discriminating dose (LD/LC₉₉).

The susceptibility of *P. xylostella* decreased considerably on rearing for 25 generations. The LC₅₀ of F₁ field collected population ranged from 56.1 to 64.0 for quinalphos, 32.0 to 32.4 for fenvalerate, 10.8 to 40.7 for carbosulfan, 50.1 to 74.7 for monocrotophos, 38.9 to 92.1 for cartap hydrochloride while the same for F₂₅ ranged from 0.5 to 3.4, 10.0 to 13.9, 0.7 to 3.1, 18.6 to 23.0 and 0.5 to 1.1 µg/ml respectively in different bioassay methods.

The extrapolated discriminating doses (LD/LC₉₉) based on the LDPM of F₂₅ population was 4.0, 5.0, 115.0, 140.0 and 3.0 µg/ml for carbosulfan, cartap hydrochloride, fenvalerate, monocrotophos and quinalphos respectively in vial assay. The same for spray tower assay was 20.0, 10.0, 170.0, 170.0 and 10.0 µg/ml while larval dip assay method gave 15.0, 10.0, 130.0, 150.0 and 10.0 µg/ml respectively for carbosulfan, cartap hydrochloride, fenvalerate, monocrotophos and quinalphos.

The assay methods adopted were comparable to each other, as the correlation among them was of high order, the coefficient being 0.99 in respect of vial to spray tower and vial to larval dip. The larval dip assay, though easier and economical, does not facilitate treatment in batches as the larvae get clumped up. Therefore, vial assay is suggested for adoption.

19-069

TISSUE-SPECIFIC ACCUMULATION PATTERNS OF PB, CD, CU, ZN, FE, AND MN IN WORKERS OF THREE ANT SPECIES (FORMICIDAE, HYMENOPTERA) FROM A METAL POLLUTED SITE

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In any quantitative survey of heavy metal accumulation in terrestrial arthropods conducted so far, ants of the subfamily Formicinae showed very high metal body burdens. Metabolic requirements of essential metals and physiological strategies to manage excess of the available metal pool in contaminated environments cause a non-uniform distribution of the elements in the tissues. Because of the beneficial role ants play in terrestrial ecosystems, the welfare of such keystone species is of general importance.

The present study investigated the concentrations of Pb, Cd, Cu, Zn, Fe, and Mn in the tissues of *Formica pratensis* RETZ., *Formica rufa* L. and *Camponotus ligniperda* (LATR.). Ants were collected in the vicinity of a closed down lead/zinc smelter, which was in operation from 1495 to 1992 in Arnoldstein, Austria.

The highest metal levels were almost always found in the midgut, followed by the hindgut and the Malpighian tubules. The mandibles appeared as a target site of Zn accumulation. Levels of Pb, Cu and Fe in the ovarioles were very high in all species. A comparison of the metal concentrations among the investigated exocrine glands revealed highest metal levels in the Dufour gland and the labial gland.

Significant differences between the species and within the metals occur. However, the degree and the history of pollution at the sampling site is of major importance and should be considered. Finally, results were discussed in the light of ecotoxicological literature.

19-068

DISPOSAL OF WASTE PESTICIDES AND RECYCLING OF PESTICIDE CONTAINERS

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A four-year project has been completed to dispose of 670 tons of waste agricultural pesticides. Collection days were held at 16 regional collection sites. Chemical disposal was conducted in the State of Arkansas by a commercial contractor in a high heat furnace. Ash residues were sealed in glass containers and placed in an approved landfill.

Educational materials were developed to inform the agricultural community about safety techniques for the collection, transportation and disposal of waste pesticide. A grant of \$5.3 million was used to support proper disposal activities. The objective of the program was to improve environmental conditions by reducing the burying and dumping of illegal waste. The Texas County Clean Up program has recycled 400,000 pounds of empty pesticide containers during the 1994 and 1995 crop seasons. The program also recycles tires, batteries, waste oil and filters.

19-070

The effects of Dorwant oil spraying on Pomegranate aphids and mites

by : S. A. Mirkarimi

High population of aphids and mites on young shoots, leaves, buds and small fruits cause considerable damage in pomegranate orchards.

In an attempt to investigate about none toxic chemical method for integrated control of these pests, 3 and 2 percent dormant oil was applied in mide march and late June respectively. Samples of aphids, mites as well as crops harvested in cheks and treated plots were compered and evaluated statistically. These investigations revealed that oil sprays reduce aphids and mites and increased fruit production up to 25 percents.

The population changes of aphids mites were investigated and the obtained data were statistically analysed and related histograms were drwan. The results indicated that, oil treatment decreased the population of mites and aphids and increased the fruit production.

19-071

SOLID PHASE EXTRACTION, MATRIX SOLID PHASE DISPERSION AND SOLID PHASE MICRO EXTRACTION TECHNIQUES FOR THE QUANTIFICATION OF ORGANOCHLORINE PESTICIDE RESIDUES IN BIOLOGICAL MATRICES.
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Quantification of organochlorine pesticide residues at parts per billion levels in animal matrices is routinely performed to monitor food supplies, monitor the effectiveness of environmental clean-up efforts and for numerous wildlife studies. Traditional approaches involve solvent extraction and quantification by gas chromatography with electron capture detection (GC-ECD). Unfortunately, this widely used approach generates large quantities of hazardous waste. Also, co-extracted lipids frequently have deleterious effects on chromatography. Reversed phase solid phase extraction and matrix solid phase dispersion were combined with GC-ECD and evaluated for the quantification of organochlorine residues in lipid rich animal matrices. When compared to solvent extraction, both methods gave a cleaner extract resulting in improved chromatography and increased sensitivity. The matrix solid phase dispersion technique resulted in extremely clean extracts, excellent precision and sub parts per billion limits of detection. Comparable results were achieved using solid phase micro extraction for the analyses of organochlorine pesticide residues in animal serum and urine samples. Furthermore, extraction and clean-up via solid phase matrix dispersion and solid phase micro extraction resulted in the generation of minimal quantities of hazardous waste.

19-073

FURTHER EXPERIMENTS ON THE BIOACTIVITY OF PLUMBAGINOIDS AGAINST THE COTTON STAINER; *DYSDERCUS KOENIGII* FABRICIUS (HETEROPTERA : PYRRHOCORIDAE)

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Further studies on the bioactivity of plumbagin and analogues, collectively termed, plumbaginoids, were carried out on the nymphs and adults of the cotton stainer, *Dysdercus koenigii* F. Plumbagin (2-methyl 5-hydroxy, 1,4-naphthoquinone) caused more mortality of young (0-day old) last instar nymphs than the 6 day old last instar ones. The lethal dose of plumbagin for 50 % nymphal mortality was about 20 µg for 0 day and >30 µg for 6 day old last instar nymphs. Besides, the inhibition of growth of the last instar nymph by plumbagin was dose dependent especially in younger nymphs. Plumbagin was more active than juglone (5-hydroxy 1,4-naphthoquinone) and lawsone (2-hydroxy 1,4-naphthoquinone), suggesting undesirability of hydrophilic substituents at 2 and 5 positions of 1,4-naphthoquinone ring. Little variation in the bioactivity of plumbagin from different sources was found when tested against 0 and 6 day old last instar nymphs. Only the highest dose of 50 µg of piperonyl butoxide synergized the activity of plumbagin (5 µg) against 0 day old last instar nymphs. Plumbagin adversely affected mating behaviour and fecundity of the adult females. The bioactivity of plumbagin is discussed in view of its reported action on neuroendocrines and integument.

19-072

The Investigation of Effect of some Insecticides on, Pomegranat - Scarabelds In Varamin In 1993-94.

Abstscat :

In some parts of the district of Varamin, some of Scarabeidae cause large damages and they play the important role of decrease in the yield rate. We took some pictures of those harms and damages. Their nutrient consists of : Sympodial roots, completely and also the skin of the main thick roots. These insects produce one generation, per-3 years.

In examining the ways to control of them, we found the effective usage in Diazinon G. 5% , 20 Kg/ha , Lindane wp 5.7% 5 Kg/ha , Sevine wp 85%, 4Kg/ha in the soil.

In 1993, by taking some samples of the different parts of the pomegranate gardens. Soil was tested for the movement of Scarabeide-larves in different depths and figures were illustrated.

In 1994, wetried out these kinds of insecticides. In an experimental of project, blocks located randomly, were applied with seven treatments and four series. Each inecticide was used at two different times, one; on 4 April, when worms came up to the surface of the earth, the other one, on 20 May, when they were in the stage of development into pupa.

The yield rate of pomegranate in each section, in fall, was so useful, in our calculation. of course, in taking samples and drawing the figure of it, we had also in mind for number of living larvae and pupae, a week after spraying; Because these things were justifying the results of the project.

The toxins experiment, were consirted of granul Diazinon 5%, Lindan 57% and sevin 85% powder forms in suspension; and we got the best results from the average of Diazinon, on 20 may.

In order to classify the average of insecticidal effects, we applied a way, called Duncan. The results of this project, in the planning of the integrated control along with the other controlling produres, will be employed.

19-074

BIOLOGICAL ACTIVITY AND RECEPTOR BINDING OF NONSTEROIDAL ECDYSTEROID AGONISTS IN COLORADO POTATO BEETLE LARVAE

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Toxicity assays demonstrated a moderate susceptibility of last-instar larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, for the first nonsteroidal dibenzoylhydrazine-based ecdysteroid agonist, RH-5849, since LC₅₀ reached 1.8 mg/l; for tebufenozide, LC₅₀ was >100 mg/l. Intoxicated larvae showed external signs of premature moulting within 12 h of treatment leading to double head capsule formation. Shortly afterwards, larvae died in a double cuticle without signs of normal shedding of the old cuticle. Electron microscopic analysis of treated larvae revealed a forced, untimely synthesis of a new, however incomplete cuticle via epidermal cell activation. Furthermore, the receptor binding affinity of the natural insect moulting hormone, 20-OH ecdysone, and the nonsteroidal ecdysteroid agonists was tested on *in vitro* cultured mesothoracic imaginal wing discs of last-instar larvae. The current data suggest that the specific ecdysteroid-mimicking activity and insect selectivity of such dibenzoylhydrazine-based compounds is related to true, however selective, binding on the ecdysteroid receptors of the epidermal cells. As a consequence, the new chemistry and mode of action of such compounds open up new avenues in the control of various agriculturally important insect pests, even non-Lepidoptera.

19-075

Glutathione S-transferase (GST) isozymes were purified and isolated from larval midguts and fat bodies of the fall armyworm (*Spodoptera frugiperda*) using a 3-step procedure involving ammonium sulfate fractionation, followed by affinity chromatography on a GSH-agarose column and nondenaturing polyacrylamide gel electrophoresis. The midgut possessed 5 isozymes, namely, MG GST-1, MG GST-2, MG GST-3, MG GST-4 and MG GST-5, all of which were heterodimers with subunit molecular weights of 26,700 to 30,000. The pI values ranged from 4.6 to 6.0 among these isozymes. No qualitative difference in isozyme composition was observed during larval development. The fat body contained 3 isozymes, namely, FB GST-1, FB GST-2 and FB GST-3, all of which were believed to be homodimers with subunit molecular weights of 20,100 to 29,000. The pI values ranged from 4.4 to 6.5 among these isozymes. Using isozyme-specific antisera as probes, MG GST-2, MG GST-3, FB GST-2 and FB GST-3 were found to be immunologically related. MG GST-2 and MG GST-3 were also immunologically related to the GST from larval Malpighian tubules of fall armyworm. Among the corn earworm, tobacco budworm, beet armyworm and cabbage looper, MG GST-3 was immunologically related to GSTs from all of these species except the cabbage looper, whereas MG GST-2 was immunologically related only to the GST from the beet armyworm.

19-077

SUBLETHAL EFFECTS OF TWO PESTICIDES ON SURVIVAL AND REPRODUCTIVE BEHAVIOR OF THE CODLING MOTH, *CYDIA POMONELLA* L. (LEPIDOPTERA: TORTRICIDAE)

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Sublethal effects of carbaryl, an insecticide and growth regulator, and cyhexatin, an acaricide, on survival and reproductive behavior of the codling moth adults, and the movement of neonate larvae, were studied under laboratory conditions. Depending on the concentration used, both pesticides resulted in high adult mortality. Exposure of the codling moth adults to most doses of carbaryl significantly reduced total oviposition. The females exposed to these concentrations, however, laid significantly more eggs during the first 24 h of exposure. While in all treatments ca. 50% of the eggs were laid on the side of the oviposition substrate, the spatial distribution of the eggs laid under the lid and on the floor was concentration dependent. Although exposure to cyhexatin, at the lower rates, increased the frequency of copulation, females exposed to all concentrations of carbaryl copulated less frequently than control. Similar effects were observed when the number of spermatophores per female were considered. In contrast to carbaryl, which exhibited a high ovicidal effect at all concentrations, the ovicidal activity of cyhexatin was low. A large number of the eggs exposed to this pesticide could complete their embryonic development, hatching at a high rate. While carbaryl killed the neonate larvae at all concentrations, cyhexatin acted as a larvicide at the highest two rates only.

19-076

DISLodgeABLE RESIDUES OF METHAMIDOPHOS IN TOMATO CROPS IN BRAZIL.

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The objective of this study was to evaluate the behavior of dislodgeable residues of methamidophos on leaves, fruits and soil of tutored tomato crops.

The experiment was conducted in a representative region in the State of São Paulo, Brazil. It consisted of four treatments (with four replicates): (1) one application of methamidophos (Tamaron BR) at single rate of 60 g a.i./100 l of water, (2) one application at double rate of 120 g a.i./100 l of water, (3) four applications at single rate, and (4) control. The leaf, fruit and soil samples were taken one day before the combined application, and then at 0, 1, 2, 3, 5, 7 and 14 days after that one.

Methamidophos dislodgeable residues were extracted with Surten aqueous solution (sodium dioctylsulfocinate - 70%); the extracts were partitioned in a mixture of 15% methanol in ethyl acetate and quantified by gas chromatography, using a flame photometric detector. Foliar dislodgeable residues of methamidophos in the treatments had degradation half-lives ranging from 0.7 to 2.4 days, and in the soil from 1.3 to 2.9 days, showing that residues in the soil are more persistent than those in the leaves.

Dislodgeable residues on fruits were detected only on 0 and 1-day old samples, and they decreased from 0.19 to 0.05 µg/cm², 0.27 to 0.06 µg/cm², and 0.11 to 0.05 µg/cm², respectively in the treatments with one application at single rate, one application at double rate, and four applications at single rate. These results show that dislodgeable residue degradation in fruit is relatively fast.

19-078

USE OF INSECTICID GRANAULE, AN INITIAL STEP TOWARD

INTEGRATED CONTROL OF CEREAL SUNN PEST *Eurygaster integriceps* PUT. (HET. SCUTELLERIDAE) IN IRAN.

M. ESMAILY, K. TALEBI, K. SEPEHR

Areal spray of about 800,000 hectare, per year against sunn pest causes numerous damages to living environment including complete destruction of beneficial arthropods both, insect pollinators, Parasitoids and Predators.

In a 4 years (1991-1995) attempts to reduce these hazards, on the basis on geotactic behavior of this pest, granular formulation of Fenitrothion 1kg.ai. and Diazinon 2kg a.i. Per hectare were applied effectively with significant reduction of honey bees and other pollinators mortality, and 50 to 70 per cent saving of scelionid and tachinid Parasitoids of sunn pest. Granule broad casting, enabled pilots to fly with higher elevation which increases working efficiency and reduces danger of fatal accidents.

19-079

FATE OF INSECTICIDES, FENITROTHION AND CARBARYL, IN FOREST SOIL AND THEIR DEGRADATION BY SOIL BACTERIA
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Fenitrothion (MEP) and carbaryl (NAC) have been used widely in Japan to control Japanese pine sawyer *Monochamus alternatus* which is a vector of pine wilt disease-agents. The fate of these insecticides in forest soil after aerial application was investigated by gas chromatography (GC) analysis. A majority of MEP (ca. 80%) in the soil disappeared on 30 days after application, although MEP in soil was still detectable slightly on 240 days. Several species of bacteria were isolated from the forest soil and were assayed for their MEP-degraded ability. Two strains of *Bacillus* sp. degraded ca. 50 % and ca. 80% of MEP in the inorganic-salt liquid medium on 5 days and 25 days after inoculation, respectively. The GC-mass spectrometry (GC-MS) analysis revealed that its major degradation metabolite is amino-fenitrothion and other minor metabolites are acetyl-amino-fenitrothion and 3-methyl-4-nitrophenol.

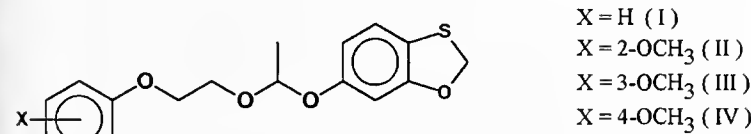
Two strains of gram-negative and rod bacterium harbored the excellent NAC-degrading ability. The strains degraded completely and rapidly NAC in the liquid medium on 10 days after inoculation. As its main degradation metabolite, 1-naphthol was detectable with GC-MS analysis. These insecticides-degrading bacteria may be available to restore the environments contaminated with insecticides applied in forests.

19-081

EFFECTS OF THREE NEW JUVENILE HORMONE ANALOGUES ON *TENEbrio MOLITOR* L. PUPAE
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Since 1974 we have been engaged in researches on new juvenile hormone analogues (JHA) with the aim to search compounds dangerous for insects only and respectful for the environment.

6-[1-(2-phenoxyethoxy)ethoxy]-1,3-benzoxathiole (I) and three new derivatives of 6-hydroxy-1,3-benzoxathiole, differing from I for the introduction on the phenyl of a methoxy group in positions 2- (II), 3- (III), and 4- (IV), respectively, were synthesized and applied on 0-24 hours old *Tenebrio molitor* L. pupae to evaluate their juvenilizing activity.



The products were applied topically on the last three urosternites at the doses of 1, 10, and 100 µg contained in 1 µL of acetic solution. For each dose of each product, 4 groups of 10 pupae have been tested; pupae treated with 1 µL of acetone and pupae not treated were used as controls.

The results of the biological tests proved that, in comparison with I, the compound II is slightly less active, the compound IV is moderately more active, the compound III is much more active.

19-080

EVIDENCE FOR A SUBSTRATE-BORNE SEX PHEROMONE AND EFFECT OF INSECTICIDES ON MATE FINDING IN PARASITOID WASPS (*TRICHOGRAMMA BRASSICAE*)
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While the use of chemical cues in host location is quite well known in parasitoid insects, little is known about their role in sexual interactions. In this work, the role of a sex pheromone was investigated in the egg parasitoid *Trichogramma brassicae*, and the evidence for a substrate-borne sex pheromone was demonstrated. Moreover, evidences that insecticides interact with communications using sex pheromone were obtained. Virgin females (<1 or 5 days old) deposited chemicals that were attractive for males whatever their mating status. Male response decreased over time but was always significant for the 8 min of video-tracking, even when male response was tested 15 min after the end of marking. Furthermore, the response of males surviving a LD30 of Chlorpyrifos (organophosphorus insecticide) was drastically decreased. Males surviving the LD30 spent only 66% of their moving time in the female-marked area vs 83% in controls. The difference was highly significant (p< 0.001) even when considering treated males with a total moving corresponding to that of controls. Then Chlorpyrifos decreases mating chances for virgin females searching for oviposition sites, and it also decreases mating chances on emergence sites. Adaptive issues of both the substrate-borne pheromone and its interaction with insecticides are discussed.

19-082

COMPARING INSECTICIDE-RESISTANT AND INDIGENOUS STRAINS OF THE PREDATORY MITE *AMBYSEIUS FALLACIS* FOR FIELD EFFECTIVENESS AGAINST *PANONYCHUS ULMI*, *ACULUS SCHLECHTENDALI* AND *TETRANYCHUS URTICAE* IN APPLE ORCHARDS
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Two strains of the mite predator *Amylyseius fallacis* were released in 1994 and 1995 in two different apple orchards in Quebec, Canada, to compare their relative effectiveness and compatibility with chemical protection spray programs. At each site, both the organophosphate and pyrethroid resistant strain, and the indigenous strain were released (100 per tree) in six tree plots ca. one week before spider mite populations reached an average of five motile forms per leaf. Populations of *A. fallacis*, *Tetranychus urticae*, *Panonychus ulmi* and *A. schlechtendali* were monitored weekly on apple foliage from June to September. Two releases were needed both years in all plots. No significant differences were observed between populations of *A. schlechtendali* or *P. ulmi* developing in presence or absence of *A. fallacis*. *T. urticae* populations remained lower in treatment plots than in control plots. Predator populations were severely affected by most of the insecticides applied on trees during the observation period. No significant differences were either observed between the two strains regarding their effectiveness or their compatibility with pest management programs used.

19-083

TISSUE-SPECIFIC EXPRESSION OF GLUTATHIONE S-TRANSFERASE ISOZYMES IN FALL ARMYWORM LARVAE

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Glutathione S-transferase (GST) isozymes were purified and isolated from larval midguts and fat bodies of the fall armyworm (*Spodoptera frugiperda*) using a 3-step procedure involving ammonium sulfate fractionation, followed by affinity chromatography on a GSH-agarose column and nondenaturing polyacrylamide gel electrophoresis. The midgut possessed 5 isozymes, namely, MG GST-1, MG GST-2, MG GST-3, MG GST-4 and MG GST-5, all of which were heterodimers with subunit molecular weights of 26,700 to 30,000. The pI values ranged from 4.6 to 6.0 among these isozymes. No qualitative difference in isozyme composition was observed during larval development. The fat body contained 3 isozymes, namely, FB GST-1, FB GST-2 and FB GST-3, all of which were believed to be homodimers with subunit molecular weights of 20,100 to 29,000. The pI values ranged from 4.4 to 6.5 among these isozymes. Using isozyme-specific antisera as probes, MG GST-2, MG GST-3, FB GST-2 and FB GST-3 were found to be immunologically related. MG GST-2 and MG GST-3 were also immunologically related to the GST from larval Malpighian tubules of fall armyworm.

19-085

THE STUDY OF MICROSOMAL OXIDASES OF MIDGUT OF CORN EARWORM *HELIOverpa armigera* (LEPIDOPTERA: NOCTUIDAE)

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R.O.C.

Corn earworm, *H. armigera* is a very important agricultural pest on corn, sorghum, ornamental flowers, tomato, pea and cucumber etc. in Taiwan. The extensive selection pressure from insecticide application the corn earworm would be expected to develop the insecticide resistant strain. The character of microsomal oxidases in corn earworm should be examined for the analysis of insecticide resistance mechanism and the strategy of insecticide application could be planned. The biochemical characteristics of microsomal oxidases between the specific activity and insecticide susceptibility and enzyme inducibility in corn earworm will be investigated in this study. The microsomal enzyme of the Tainan corn earworm midgut would be assayed in this experiment. The microsomal enzyme of the activity of aldrin epoxidase was the highest (4539.2 pmole/min/mg protein), among the others activities as Biphenyl hydroxylase, Cytochrome b5, N-demethylase, Cytochrome p-450, MR O-demethylase, ER O-deethylase.

19-084

CONTACT AND *PER OS* LETHAL CONCENTRATION VALUES OF DIFLUBENZURON ON *MELANOPLUS SANQUINIPES* (F.) (Orthoptera: Acrididae).B. W. Fuller, M. A. Boetel, L. Jech¹, T. Wang & R. N. Foster¹.Department of Plant Science, South Dakota State University,
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The migratory grasshopper, *Melanoplus sanguinipes* (F.) is a serious cropland pest, and some rangeland outbreaks may include this insect and closely related melanoplinae. We used this easily reared pest and believe its susceptibility to be a good indicator for extrapolating lethal concentrations for many of our rangeland grasshopper species. Our bioassays used 3rd instar *M. sanguinipes* (F.) that received diflubenzuron via both *per os* and topical applications. Treated discs of lettuce facilitated *per os* treatments while topical dosages were applied in 1 ml aliquots of acetone to the abdominal sternites. Corrected mortality rates were subjected to probit analysis using the POLO-PC software program.

No mortality was detected in the *per os* treatment until 120 h post exposure (feeding) at which the calculated LC₅₀ was 1656 ppm (range = 789-2447) (95% CI). Responses to topical applications at 120 h resulted in LC₅₀ calculations of 31 ppm (range = 22-40). The LC₅₀ topical dose was 53 times more effective than the same *per os* dosage. Implications for field use will be presented.

19-086

METAL CONCENTRATIONS IN VARIOUS DEVELOPMENTAL STAGES OF ENDANGERED *PARNASSIUS APOLLO* (LEP.: PAPILIONIDAE) IN PIENINY MOUNTAINS (SOUTHERN POLAND).

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In last three decades rapid decline of *P. apollo* populations has been noted in Poland and some other European countries. Among the reasons of this decline there may be anthropogenic pollution. Fragmentary data so far obtained suggest, that food-plant of apollo larva - various orpine species (*Sedum spp.*) - strongly accumulate cadmium and some other metals. Observations of apollo developmental anomalies and high pupae mortality in semi-natural colony carried out in Pieniński National Park, suggested possible influence of metals also on ontogeny of this species.

So the aim of this study was to evaluate whether the insects of successive developmental stages are really exposed to excessive concentrations of various metals, particularly the highly toxic ones - cadmium and lead. Due to small number of free-living individuals the specimens for analyses came mostly from the colony. Concentrations of Cd, Pb, Zn, Fe and Cu were determined in dried samples of livelong orpine (*S. maximum*) leaves and the insects of various stages after complete wet digestion (HNO₃:HClO₄ - 4:1) by means of flame AAS.

Obtained results confirmed the suggestion, that some metals, particularly Cd and Pb, can be a stressing factor to apollo larvae and also to adults. *Sedum* leaves contained high concentrations of cadmium, lead and zinc. These metals came probably mainly from dust fallout. Cadmium cumulated in the body with the age of larva and its concentrations were higher than in food-plant. Due to the specific feeding habit the youngest larvae are particularly exposed to lead depositing on the leaves surface. Older instars could maintain relatively constant body burden of Pb. Adults that do not eat orpine leaves also contained high concentrations of the metals. It was interesting, that the „wild” insects were significantly more contaminated than the individuals from colony.

19-087

THE INFLUENCE OF CADMIUM AND SELENIUM ON DEVELOPMENTAL ACTIVITY PATTERN OF CARBOXYL-ESTERASES IN HEMIMETABOLOUS INSECTS.

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University of Silesia, Department of Human & Animal Physiology, Bankowa 9, Katowice, Poland.

Carboxylesterases (CarE) are widely distributed group of mainly cytosolic enzymes, that metabolise many endogenous as well as exogenous compounds. Hence, disturbances of their activities may severely affect detoxifying abilities of an organism. They are particularly important for developing individuals. Earlier studies have shown, that cadmium - one of the most toxic metals - stimulated or inhibited, depending on the dose, the enzymes activity in midgut tissues of last larval instar and adults of house cricket (*Acheta domesticus*), and young adult madagascar cockroach (*Gromphadorhina portentosa*). The aim of this study was to determine whether selenium ($0.01 \mu\text{mol Se} \cdot \text{g}^{-1}$ of dry weight feed), considered as a cadmium antagonist, would protect these insects from sublethal poisoning with Cd ($0.4 \mu\text{mol} \cdot \text{g}^{-1}$ of dry weight feed) during later stages of their ontogeny.

Female crickets were intoxicated from 20th day after hatching, at least for 2 weeks, and female cockroaches - for 7 - 9 weeks. CarE activities were measured in crude homogenates obtained from midgut using α -naphthyl acetate (α NA) as substrate in 1st, 7th and 50th day after final molt of *G. portentosa* larvae. The activity in crickets was measured in 1st, 5th and 9th day of last larval instar and from 1st to 30th day of adult stage with 5-days intervals.

In the given dose cadmium stimulated the activity of CarE in midgut of both species, but in cricket it occurred during the whole examined period, while in cockroach it appeared only in the last age-group. Selenium itself did not cause marked changes of the enzymes activities in these species but given simultaneously with cadmium significantly decreased its effects. However, the insects differed in their response to these elements. Protective action of Se against cadmium depended on the age in cockroach, while no such relation has been observed in crickets.

19-089

EFFECT ON THE MORTALITY OF THE OLIVE-TREE ENTOMOFAUNA CAUSED BY ADULTICIDAL AND LARVICIDAL TREATMENTS USED TO CONTROL THE OLIVE-FLY: *Bactrocera oleae* (Gmel.) (DIPTERA, TEPHRITIDAE). TESTS CARRIED OUT IN THE THREE-YEAR PERIOD OF 1991-93 IN THE OLIVE-GROWING ENVIRONMENT OF NORTHERN LAZIO (CENTRAL ITALY).C. Pucci¹, A.F. Spanedda¹, A. Forcina², A. Giovagnoli³¹Dipartimento di Protezione delle Piante, Università della Tuscia, Viterbo (Italy)-²Dipartimento di Statistica, Università degli Studi, Perugia (Italy) - ³Istituto di Statistica, Università degli Studi, Bologna (Italy)

A comparative test was carried out in the olive-growing area of Canino (Northern Lazio) on the mortality, with regard to the olive-tree entomofauna, determined by the adulticidal and larvicidal treatments normally used against the *Bactrocera oleae* (Gmel.).

The experiment was carried out and repeated for 3 consecutive years, in a 2 ha olive-grove, in which 20 homogeneous plants of the same age were selected from the Canino cultivar, subdivided into 5 thesis with 4 repetitions and distributed at random, taking care that the sample plants were not adjacent to one another.

The insecticides compared were:

Fenthion + proteinaceous lure (adulticidal), Deltamethrin + proteinaceous lure (adulticidal), Dimethoate (larvicidal).

The methodology adopted for detecting the data was that suggested by IOBC in "Standard methods to test the side-effects of pesticides on natural enemies of insect and mites".

Under the projection of the canopy of each plant 4 collectors were located to correspond with the four cardinal points.

The insects which were gathered, distinctly per plant and per collector, were counted and separated by Systematic Order.

With the statistical analysis of the data, based on the adaptation of several log-linear models, the different effect on the mortality produced by the larvicidal and adulticidal treatments on the elements of the different Systematic Orders was assessed, together with their relative density in distinct periods of the year.

19-088

AN INSECTICIDAL CYCLOPEPTIDE RESPONSIBLE FOR BRUCHID RESISTANCE IN A WILD MUNGBEAN *M. Ishimoto*¹, *F. Sugawara*², *K. Kitamura*¹¹ National Agriculture Research Center, Tsukuba, Ibaraki, Japan-2 The Institute of Chemical and Physical Research (RIKEN), Wako, Saitama, Japan

Seeds of grain legumes in the genus *Vigna*, such as cowpea (*Vigna unguiculata*), mungbean (*V. radiata*) and azuki bean (*V. angularis*) are staple foods in many countries. Post-harvest damage of the seeds by a group of bruchids (Coleoptera:Bruchidae) is quite extensive. Previously, a wild mungbean strain (TC1966) which possesses bruchid resistance was identified. This resistance is controlled by a single dominant locus; however, the principal factor for the resistance had never been characterized. Here we report on a cyclopeptide, named vignatic acid ($\text{C}_{30}\text{H}_{39}\text{N}_3\text{O}_7=553$), which has been identified as a principal factor conferring the bruchid resistance. This is the first example of a plant peptide which has insecticidal activity.

19-090

A STUDY ON THE MODE OF ACTION OF CARBOSULFAN BY ACTION OF CYTOCHROME P450 VIA BIOACTIVATION PROCESS. *J. H. HUR*, *D. S. HAN*, *K. W. YANG*¹, *S. J. LEE*. Department of Agricultural Chemistry, Kangwon National University, 200-701, Korea.

This study focused on understanding a proposed hypothesis that carbosulfan must be converted to a more potent anticholinesterase to show its toxicological properties in biological systems.

The bimolecular inhibition rate constants of carbosulfan to AChE and BuChE were approximately $10^3 \text{M}^{-1} \text{min}^{-1}$ which was low enough not to inhibit the ChEs effectively. The potency of carbosulfan as an inhibitor of AChE and BuChE was increased when carbosulfan and ChEs were incubated with microsomes fortified with NADPH compared to the microsome alone. Piperonyl Butoxide (PB), when added to these coupled systems, greatly reduced the inhibition of both enzymes by blocking a bioactivation process.

The I_{50} value of carbosulfan for *in vivo* mouse brain AChE was 14mg/kg, while this value was altered to 162mg/kg when PB was pretreated. Rat blood AChE and BuChE were similarly inhibited *in vivo* by carbosulfan administered i.p. at 130mg/kg. Maximum inhibition was observed at 8hr for AChE and 1hr for BuChE. When PB was pretreated, more inhibition was observed than that of the control.

These results showed that the bioactivation of carbosulfan is essential for showing its toxicological action and cytochrome P450 must be an important enzyme which possibly participated in the bioactivation process.

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19-091

DOSE RESPONSE ANALYSIS OF FENTHION TOXICITY IN TWO BRAZILIAN POPULATIONS OF *Anastrepha fraterculus* (DIPTERA: TEPHRITIDAE)I.B.M. Da Cruz¹, E. Humeres², C. Bica¹, A.K. Oliveira¹

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Anastrepha fraterculus (Wiedemann), the South American fruit fly, highly polyphagous and known to attack dozens of cultivated and wild fruits (Malavasi et al. 1980; Norrbom & Kim 1988). In southern Brazil, it causes severe fruit damage in apple orchards. We estimated basic toxicity data for two *A. fraterculus* populations and general handling methods for contact and feeding bioassays in F1 of two south Brazilian field populations of the *A.* (N= 3.780). The slope are the same between males and females of populations studied. Contact bioassay was more effective ($LD_{50} = 1.11$ ppm) when compared to feeding bioassay ($LD_{50} = 20.51$ ppm). In these two different experiments males and females were similar in the responses. The results obtained could help the implementation of a rational *A. fraterculus* management integrated of pest (MIP) that is not yet made today and are related with biology, populational dinamic of South American fruit fly. Grants and Fellowships: CNPq, ABPM.

19-092

PHYSIOLOGICAL EFFECTS OF CHLORFLUAZURON ON THE REPRODUCTIVE ACTIVITY IN *SPODOPTERA LITURA*

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It is acknowledged that benzoylphenyl urea compounds inhibit chitin synthesis in insects and thus they disturb molting process and metamorphosis. Also benzoylphenyl urea compounds are known to affect the reproduction of insects. We investigated the reproductive effects of chlorfluazuron in *Spodoptera litura* topically applied at larval stage. When chlorfluazuron was applied to newly molted 5th instar larvae at a sublethal dose (LD_{10} , 31.2ng/larva), it was observed that the fecundity of the resulting adults as well as the hatching rate of their eggs was suppressed. The hatching rate of eggs oviposited by an untreated female copulated with a treated male was suppressed to the similar degree to that of eggs oviposited by a treated female copulated with a treated male. In males of *S. litura*, testes develop during the larval stage and attain complete differentiation in the pupal stage. Larval treatment of chlorfluazuron resulted in the reduction of the size of testes, the reduction of the number of sperm bundles, the delay of spermatogenesis, and the reduction of testes ecdysteroids. Thus, it was considered that the suppression of testis ecdysteroids titers by chlorfluazuron caused to decrease the number of sperm bundles and the sperm volume.

19-093

ACTIVITY OF AN ACETONIC EXTRACT OF *TRICHILIA HAVANENSIS* (MELIACEAE) ENDOSPERM ON *SPODOPTERA EXIGUA* LARVAEJ. Lopez-Olguin¹, M. C. de la Torre², E. Viñuela³, P. Castañera¹

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A bioassay-guided, silica gel column fractionation of an acetone endosperm extract of *Trichilia havanensis* Jacq. yielded seven fractions. Four of them were pure compounds, identified as the limonoids: azadirone, trichilenone acetate, 14,15-deoxyhavanensin-3,7-diacetate and 14,15-deoxyhavanensin-1,7-diacetate. Short-term choice and no-choice feeding bioassays of each fraction (at 1000 ppm) showed antifeedant activity against fifth instar larvae of *Spodoptera exigua* in the most polar fraction only.

Rechromatography of the active fraction resulted to be a mixture of two compounds. Both compounds were isolated and their structures, established by NMR, correspond to 14,15-deoxyhavanensin-1,7-diacetate and havanensin 1,7-diacetate (1:2 w/w). This mixture exhibited antifeedant activity (at 1000 ppm) in short-term choice and no-choice feeding bioassays. Interestingly, when both compounds were singly evaluated, havanensin 1,7-diacetate was the active one, though its activity was lower than that found in the mixture.

19-094

LABORATORY TOXICITY TEST OF SEVERAL NATURAL PESTICIDES ON *ALEURODICUS DISPERSUS* RUSSELL (HOM.: ALEYRODIDAE) THE SPIRALLING WHITEFLYI. Zoltan¹, E. Kiss Ferencné¹, I. Kajati², C. Budai¹, R. Torres³, M. Hernández-García³, E. Hernández-Suárez³, A. Carnero³.

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The spiralling whitefly *Aleurodicus dispersus* was discovered in Canary Islands in 1964. However in the two last years it has been the first problem in the gardens of the coast areas of the Canaries.

The pesticides of Hungarian patent VEKTAFIG[®] A 1%, VEKTAFIG[®] R 1%, BIOSECT[®] 2% and TIOSOL[®] 1% were selected by their natural origin and the low toxicity on the beneficial insects and the human being.

The side effect of this natural pesticides on the pest *A. dispersus* were tested in laboratory going on the guidelines of the IOBC-Working Group of pesticides and beneficial Arthropods.

The best results were obtained with VEKTAFIG[®] A 1% and BIOSECT[®] 2% on the different development stages of the insect.

19-095

EVALUATION OF INSECTICIDE FOR *PIERIS BRASSICAE* (L) CONTROL ON CABBAGE IN MEGHALAYA, INDIA.

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The recommendation of chemical for control of cabbage caterpillar eleven insecticides, malathion 50 Ec @ 1 kg. a. i/ha, chlorpyrifos 25 Ec @ 0.5 kg. /ha, monocrotophos 36 Ec @ 0.5kg./ha, fenvalerate 20 Ec @75 gm./ha, deltamethrin 2.8 Ec @ 12.5 gm/ha, cypermethrin 10 Ec @ 75 gm/ha, endosulphon 35 Ec @ 0.7 kg./ha, fenitrothion 50 Ec @ 0.8 kg./ha, quinalphos 25Ec @ 0.5 kg./ha, diflubenzuron 25 wp @ 75 gm/ha were tested in 3 replicated 2 trials on plots 3 x 2.5 m containing 20 plants at pre and post headed stage, when on them larval density was $14.67 - 23.67 = (\sqrt{x} + 0.5)$ in 1991-92.

The control status and yield data were analysed following DMRT (Duncan's multiple range test), the residual tolerance and cost benefit ratio (CBR) calculated. Evaluation showed 100% (99%) control by fenvalerate with 99% safety to natural enemies and mammal (LD 50,200-451), 97.73% (99%) by deltamethrine (LD 50, 128-138), 96.8% (98%) by cypermethrine (LD 50, 242-303), 85.2% (100%) by diflubenzuron (LD 50, 2150).

The CBR of fenvalerate yielded 172.13 q/ha with net gain of 96.8 q/ha (Rs. 29,558.00) CBR 1:43, deltamethrine and cypermethrine (Rs. 6,842.00 /ha) CBR 1:33, 1:32 and diflubenzuron (Rs. 10,481.00 /ha) CBR 1:44 . Therefore on economic gain, safety and environmental friendly reasons fenvalerate, deltamethrine, diflubenzuron and cypermethrine recommended to farmers.

19-097

TACTICS FOR MANAGEMENT OF INSECT RESISTANCE TO INSECTICIDES FROM PAST EXPERIMENTAL DATA

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Insect resistance to insecticides is very difficult matter for management of insect pest and meaning matter in the sciences include insect physiology, ecology, genetics, toxicology and biochemistry. Missing of excellent insecticides by occurring of resistance is very serious problem not only for industry also public sector and farmer. Because they can not establish insect control programme and can not recommend effective insecticides to their farmer.

We have many reports on the insecticide resistance from past experimental data. Regarding on origin of resistance, we believed that the insecticide acts to select those naturally possessing factors conferring resistance and that eventually the resistance progeny become dominant in the population. It is a kind of insect adaptation and revolution to insecticidal environment. Following suggestions could proposed tactics for management of insect resistance to insecticides.

1. Insecticide resistance monitoring in key insect pests.
2. Insecticide rotation use of no cross resistance insecticides.
3. The tactics of delaying of insect resistance to insecticides with pesticide management.

The studies on insecticide resistance in the diamondback moth, the brown planthopper and the citrus red mite have been conducted. Tactics for management of these insect pests resistance to insecticides and acaricides to be discussed.

19-096

THE TOXICITY OF CYROMAZINE TO *CHIRONOMUS ZEALANDICUS* AND *DELEATIDIUM* SP.

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Cyromazine and a commercial Formulation Vetrazin were tested against *Chironomus zealandicus* and *Deleatidium* sp. Under acute conditions, the LC50 values were comparable. For *C. zealandicus* they varied according to instar from 100-400 mg per litre for second and third instars to 1000-10000 mg per litre for old fourth instars. For the one size class of *Deleatidium* tested, the value was 300-400 mg per litre. In chronic whole-of-life tests with *C. zealandicus*, cyromazine was very toxic. A maximum acceptable toxicant concentration of 17.5 ug per litre was calculated. The implications of the difference between acute and chronic results for getting environmental exposure limits with compounds like cyromazine is discussed.

19-098

DETECTION AND MANAGEMENT OF RESISTANCE TO AZINPHOS-METHYL IN *CYDIA POMONELLA* (L.)

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Cydia pomonella (L.), codling moth, has long been a serious insect pest of apples and pears in south-east Australia. From 1950, following the loss of DDT through resistance, control in commercial orchards was largely based on carbamate and organophosphate insecticides. In the last 20 years, azinphos-methyl has provided the majority of the industry with cost-effective control.

Resistance to azinphos-methyl was first suspected in 1989 when a small number of apple growers in each of the three eastern mainland states of Australia experienced control failures which could not be attributed to pesticide application factors alone. Adult bioassays subsequently diagnosed resistance to azinphos-methyl up to 6.8 fold at the LC₅₀. Alternative insecticides for control were investigated but neither chlorpyrifos nor fenoxycarb were effective where resistance levels were highest. The range of chemical alternatives is limited because of increased reliance of the Australian apple and pear industry on integrated pest management practices.

A resistance management strategy was developed for the industry and is periodically reviewed. At present the strategy relies on growers decreasing the use of azinphos-methyl through improved timing using spray warning services and field monitoring. Orchard sanitation practices are recommended to reduce the carryover of *C. pomonella* between seasons. Mating disruption has been successful in limiting the use of insecticides for *C. pomonella* control in some orchards although used alone, mating disruption has not yet proved sufficiently robust under Australian conditions to become a major component of the strategy in orchards where resistance already occurs. Mating disruption in conjunction with a modified insecticide program is under investigation as part of the resistance management strategy.

19-099

EFFORTS TO REDUCE THE LEVELS OF CYROMAZINE RESISTANCE IN THE LEAFMINER, *LIRIOMYZA TRIFOLII*, POPULATIONS IN SOME TOMATO - PRODUCING AREAS OF SOUTH AFRICA

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One of the most important pests of tomatoes in certain areas in South Africa is the leafminer *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) which was introduced to South Africa with chrysanthemum cuttings in 1983.

Serious yield losses occurred especially in areas where tomatoes are produced for 11-12 months of the year. Insecticides registered against other tomato pests were not effective because leafminer is resistant to most insecticides. The insect growth regulator cyromazine was registered in 1986 by Ciba-Geigy South Africa for leafminer control. In 1991 complaints were received of poor leafminer control in Mooketsi. Resistance of local leafminer populations was suspected. This article reports on the cyromazine susceptibility/resistance of some leafminer populations from Mooketsi and from other areas which were tested in the laboratory. Data was collected over a two-year period. A spray programme for tomatoes against leafminer resistance management was also introduced after the first data was collected. This data was compared to the second year's data, to compare the levels of resistance.

Ciba-Geigy South Africa and the Agricultural Research Council's, Roodeplaat Vegetable and Ornamental Plant Institute, was involved in this research.

19-101

PRO-ACTIVE RESISTANCE MONITORING PROGRAMS FOR AVERMECTIN INSECTICIDES IN CROP PROTECTION

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The avermectin insecticides (abamectin and emamectin) have an unprecedented potency to arthropod pests. LC90 values range between 0.001-0.2 ppm for most pests for which control is claimed. Pro-active monitoring for resistance to the avermectin insecticides has been conducted for several years. Currently, resistance monitoring programs are focused on those pests considered to be most problematic with regard to development of resistance to insecticides. These pests include twospotted spider mite (*Tetranychus urticae* Koch), leafminer (*Liriomyza trifolii* (Burgess)), Colorado potato beetle (*Leptinotarsa decemlineata* (Say)), and diamondback moth (*Plutella xylostella* (L.)). We are also monitoring for resistance to abamectin in citrus rust mite (*Phyllocoptruta oleivora* (Ashmead)). Marked differences (> 100-fold) in twospotted spider mite susceptibility to abamectin have been observed among populations. The highest levels of resistance occur on glasshouse-grown ornamentals that are subjected to intense selection pressure. However, practical resistance (i.e., field failure) has not been observed to date. In leafminers and Colorado potato beetle, extensive monitoring has found up to 16- and 15-fold differences in susceptibility to abamectin, respectively. In diamondback moth, up to 4- and 100-fold differences in susceptibility to emamectin and abamectin, respectively, were found. A review of each of these monitoring programs will be presented. The importance of pro-active resistance monitoring in resistance management programs will be highlighted.

19-100

COMBATING RESISTANCE TO IMIDACLOPRID IN WHITEFLIES BY VARIOUS PREVENTIVE STRATEGIES (HOMOPTERA: ALEYRODIDAE)
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One of the most encouraging developments in whitefly management in Imperial Valley, CA, has been the introduction of imidacloprid-formulated insecticides. Imidacloprid provides season-long control in fall vegetables and spring melons, and requires fewer treatments than with spray insecticides. This compound represents a valuable new tool in insect pest management, and as such should be safeguarded from debilitation through largescale resistance development. Therefore, resistance management programs are essential for prolonging the life of imidacloprid under field conditions. Towards this goal, we are evaluating restriction of imidacloprid treatments, alternation of imidacloprid with insecticides having different modes of action to delay resistance and the effect of immigration of susceptible individuals.

A resistant strain of *Bemisia tabaci* was established by selection with imidacloprid for approximately two years. The stability of imidacloprid resistance in this strain is under evaluation using a rotation regimen of different chemistries and also by withdrawal of imidacloprid selection. Five subsets of this resistant colony were subjected to selection with imidacloprid, bifenthrin, chlorpyrifos, amitraz and buprofezin on a continuous and rotational sequence. A sixth subset of the resistant strain was maintained with no selection. Susceptibility to imidacloprid was monitored using a hydroponic bioassay developed in this laboratory. Resistance to imidacloprid remained high with continuous selection (RR= 60-fold). Lower levels of resistance to imidacloprid were observed in whiteflies subjected to a rotation regimen with different insecticides. Resistance to imidacloprid was slow to decline initially in the absence of selection but declined considerably after a few generations. Our study indicates that resistance to imidacloprid declines when treatments are withdrawn. Additionally, resistance can be delayed under a rotational scheme in combination with different chemistries, thus prolonging the effectiveness of imidacloprid.

19-102

INSECT RESISTANCE- INDUSTRY IN PARTNERSHIP THROUGH IRAC

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Insect resistance to insecticides is a well documented and growing problem. In an age of environmental consciousness, the decreasing number of insecticides available for use constantly reduces the available options. This and the restricted number of different modes of action available requires all involved in agriculture and pest control to preserve our present weapons in the battle against insect and mite pests.

The importance of resistance is widely perceived, with input from regulators, academics, officials and farmers. Getting effective means of combating resistance is in everyone's interest and requires clear communication between all parties. Only in this way can we achieve sustainable pest control. The paper discusses practical examples of the problem, what industry (as IRAC) is doing and assesses the future collaborations which will be required to achieve this objective.

19-103

INSECTICIDE RESISTANCE DYNAMICS IN
Helicoverpa armigera (HUB) IN EASTERN U.P.
(INDIA)

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Discriminatory dose testing in 30-40 mg larvae of *H. armigera* from six locations within 70 km radius from Varanasi against cypermethrin, endosulfan, quinalphos and fenvalerate exhibited high resistance not referable to location, cropping system, and insecticide input. Regular fortnightly observations from Varanasi showed highest resistance in Oct population influx on early pigeonpea after two months of absence. A less severe resistance peak was exhibited during Feb-April. Both the peaks diluted drastically in successive months. No change of mixed function oxidase, acetylcholinesterase and carboxylesterase activity and host plants on resistance and also the fact that resistance level corrected with growth rate, suggested this phenomenon to be due to vigour tolerance. Draustic reduction of resistance in inbred population during F1 and F2, followed by nonviable egg laying during F4 and F5 and lack of genetic nature suggested this resistance to be of acquired origin. Perhaps the resistance in this innocent region is brought from distant focal areas with intense insecticide use through migration/dispersal.. This gets diluted to the level of completely susceptible population in July and August every year most of which perishes out of self sterility supporting migration.

19-105

RESISTANCE TO AZINPHOSMETHYL IN CODLING
MOTH: PATTERNS AND MANAGEMENT POTENTIAL

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Low levels of resistance to azinphosmethyl were discovered in
populations of codling moth in 1989 in California. More recent
surveys have detected 4-15 fold levels of resistance in additional
sites and counties in California. While azinphosmethyl resistance
is still at levels that do not confer field failures, growers in areas
with resistance experience difficulty controlling codling moth,
resulting in increased Guthion use. Whereas multiple
mechanisms of resistance are suspected, resistance to
azinphosmethyl has been correlated with other organophosphates,
carbamates, pyrethroids (e.g. esfenvalerate), and one insect
growth regulator. Negatively correlated cross resistance also has
been observed with chlorpyrifos and methyl parathion.
Pheromone mating disruption was initiated on a regional basis in
1993 over 308 ha of Bartlett pears as a resistance management
tool to take advantage of the high fitness cost associated with
azinphosmethyl resistance in codling moth. In 1994, initial
surveys suggested a 20% decline in mean resistance levels for the
region after 2 years of mating disruption. However, in 1995, this
pattern was reversed apparently by single widespread application
of azinphosmethyl to the project.

19-104

SUBLETHAL DOSAGES OF SOME INSECTICIDES: AN
IMPROVEMENT TO THE EFFICACY OF BIOINSECTICIDES

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Bioinsecticides based on *Bacillus thuringiensis* have a slow initial action and their efficacy very rarely reaches the degree of the efficacy of chemical insecticides. At the Agricultural faculty at Zagreb first trials to improve these deficiencies started Kovačević as early as 1958 and were continued later by other scientists (Maceljski, 1974 etc.). In the last years we tried to investigate the possibility to include the use of sublethal dosages of chemicals in the Colorado potato beetle (CPB) resistance management, because the problem of resistance of this insect to OP, OC and P insecticides is very acute in Croatia. We expected to improve the control of the CPB by using ecologically safer control methods.

By sublethal dosage we consider dosages lower than 10% of the normal dosage. Low dosages consisting from 10--50% of the recommended dosage we consider as subnormal dosages.

In our two years trials with larvae of the CPB sublethal dosages of some OP (chlorpyrifos) and P (alphacypermethrin, lambda-cyhalothrin and a combination of chlorpyrifos and cypermethrin) insecticides so as one IGR (hexaflumuron) were added to Novodor, an insecticide based on B.t. tenebrionis. The trials were conducted by using the IRAC 7 method. To the recommended dosage of Novodor, dosages containing 1-10% of the recommended dosage of the previously mentioned insecticides were added.

In most cases between the components used in trials an independent synergism was established. Some cases of antagonism were established also. The results of these trials shows a good possibilities to improve the action of the B.t. bioinsecticide by using sublethal dosage, but will be continued by including dosages of chemicals lower than 1% of the recommended dosage.

19-106

PRESENT INSECTICIDE USE PRACTICES ON COLE CROPS
IN PUNJAB (INDIA) AND STRATEGIES FOR MANAGING
MULTIPLE INSECTICIDE RESISTANCE IN DIAMONDBACK
MOTH

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The indiscriminate and intensive use of insecticides for producing bluish free heads of cole crops has resulted in emergence of multiple resistance in Diamondback moth to currently available major groups of insecticides. Cross-resistance investigations have revealed some highly promising alternate insecticides and bioinsecticides with entirely different mode of action like Cartap hydrochloride, diafenthiuron, flufenoxuron and B.t. Integration of these products with conventional insecticides by their sequential and or combined use for managing the pest population below the threshold levels so as to delay the onset of resistance would require field scale evaluation. The strategy thus formulated would take into consideration the commercial quality criteria of the grower achievable by sustained curbing of the build up of the pest population without accompanying residue hazards.

19-107

THE STRUGGLE WITH LARVAE OF BLOOD-SUCKING MOSQUITOS BY THE METHOD OF INSECTICIDES AND REGULATORS OF INSECT DEVELOPMENT (RID) INSERTION IN TURNS INTO NATURAL RESERVOIRS

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Resistance rise for two agents, effected on different stages of insect development, is less probable than for intensive applied single one. So we have developed the rotary schemes for the preparations related to RID group - juvenile hormone analogues (JHA) and chitine synthesis inhibitors (CSI) and to the groups of phosphorous organics (POC), pyretroides and bacterial insecticide preparations. The rotary schemes were formed on the strength of the resistance rise mechanisms: JHA - pyretroides; CSI - POC; CSI(JHA) - bacterial preparations. Prolongated effect (equal to 1,4-2 times for POC and 1,3-1,6 times for pyretroides and bacterial preparations) is achieved if these preparations are inserted after JHA or CSI and not on the contrary, since the combination "insecticide - JHA (CSI)" had an ordinary effect. The term of the second agent insertion is a crucial factor for the effect prolongation. This regularity was corroborated during 5 years at the 182 reservoirs at aride and temperate zones. Insertion of preparations "JHA (CSI) - insecticide" in indicated turns results in 1,5-2 times expense quota decreasing with preservation of effectiveness; that leads to reduction of the number of preparation insertions into environment and so to decreasing of ecologic load per hectare.

19-108

EXPLORING PLANT PRODUCTS POTENTIAL FOR THE MANAGEMENT OF INSECTICIDE RESISTANCE OF *HELICOVERPA ARMIGERA* IN COTTON IN INDIAGavi Gowda¹, A.Regupathy², N.J.Armes³

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Regular monitoring of field populations since 1993-94 revealed that resistance both to fenvalerate and cypermethrin was very high in all monitoring regions with situations being most severe (more than 80% survival) at fenvalerate 0.2 µg and cypermethrin 0.1 µg discriminating doses. Studies were conducted to optimize pest management components with a view to developing and insecticide resistance management (IRM) strategy for cotton in India.

Application of neem oil based formulation viz., Nimbecidine 0.03% EC and TNAU Neem 0.03% EC effected 45-70% reduction of leaf hoppers, *Amrasca biguttula biguttula* Ishida and 30-60% by reduction of cotton aphid, *Aphis gossypii* G.

Application of neem seed kernal extract (NSKE) 5%, neem leaf extract (NLE) 5%, *Vitex negundo* leaf extract (VLE) 5%, *Ipomoea* leaf extract (ILE) 5% and Pongamia oil 80 EC (PO 80 EC) at 0.2 and 0.4 % reduced egg laying by 53.6%, 52.6%, 27.8%, 4.0%, 37.5% and 69.0% respectively. Among the various oils, (sesamum, cotton, sunflower, palm, niger, custard apple, castor, neem citronella and pongamia) evaluated pongamia oil synergised well with fenvalerate and cypermethrin. The suppression of fenvalerate and cypermethrin resistance was to the extent of 80% and 59.6, respectively. The level of suppression was comparable with the effect of piperonyl butoxide, the mixed function oxidase inhibitor synergist. Other oils did not show any significant synergistic effect.

The potential of Nimbecidine 0.03% EC and TNAU Neem oil 0.03% to formulations for checking early season pests and NSKE, NLE, VLE, PO 80 EC as a practically realizable synergist for fenvalerate and cypermethrin for *H.armigera* is discussed.

19-109

INSECTICIDE RESISTANCE AND THE EC AUTHORISATION REQUIREMENTS

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ABSTRACT

Provision of information on resistance is a requirement of the pesticide registration process in the European Union (EU). An outline of how these requirements relate to insecticide resistance is provided in this paper.

Section 20

Entomophagous Insects and Biological Control

20-001

GLOBAL APPLICATIONS AND REQUIREMENTS FOR ARTIFICIALLY REARED PARASITOIDS AND PREDATORS
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The use of insect parasitoids and predators in augmentative biological control is currently limited by cost of production, storage and handling techniques, standards and procedures for measuring success, efficacy data on levels of parasitization and crop protection, regulatory requirements for permits and "labels," creative strategies for their use, and resources dedicated to research and technology transfer. Ongoing basic and applied research is overcoming many of these limitations by developing *in vitro* rearing techniques and conducting related field evaluations. This work is focused on insect parasitoids and predators, and associated cropping systems that represent promising biological control systems.

20-002

INSECT-FREE DIET TESTED FOR REARING *PERILLUS BIOCULATUS*, *PODISUS MACULIVENTRIS* AND *COLEOMEGILLA MACULATA*.

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The objective of this study was to evaluate the nutritional viability of a single empirical medium for several species of entomophages. Two pentatomids and one coccinellid were tested since an empirically based medium may prove more complete for predaceous entomophages, due to their reliance on extra-oral digestion to enhance the nutrient quality of their food source. Gel and liquid forms of the medium were used as well as rearing chambers constructed of different materials.

Newly emerged adults of *Podisus maculiventris* (Hemiptera: Pentatomidae) fed on the liquid form of the medium, laid viable eggs, and the F₁ generation developed through to adults when reared on the liquid medium, but eggs of the F₁ adults were not viable. Adults of *Perillus bioculatus* (Hemiptera: Pentatomidae), that were converted from a larval-lepidopteran diet to the liquid medium, oviposited, the eggs hatched, and the F₁ nymphs developed when reared on the liquid medium, to adults that oviposited but none of the eggs were viable. Larvae and adults of *Coleomegilla maculata* (Coleoptera: Coccinellidae) fed best on a gelled version of the medium. Only late instar larvae were tested, and they did develop through to the adult stage. The development of all three predators was slower on the empirical medium than on an insect-based diet. Seasonal variations (e.g. summer vs. winter), medium delivery system, and chamber composition (e.g. paper vs. plastic) may impact the observed rate of development. Although limitations are evident with this insect-free empirical medium, it may have the potential of serving as a starting medium for predaceous entomophages.

20-003

ARTIFICIAL DIETS FOR REARING A PREDACIOUS COCCINELLID *MENOCHILUS SEXMACULATUS* FABRICIUS

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Menochilus sexmaculatus is the most common and intensively studied species of coccinellid predator in Malaysia. Currently the larva and adult predators can be successfully reared on aphids of various species. However, rearing the predators using artificial diets has only been attempted recently. A diet consists of chicken liver, yeast and sucrose has been formulated, tested and found suitable for rearing larvae and adult predators. Adult predators (males and females) seemed to live longer when fed on artificial diets as compared to diets of aphids (*Aphis craccivora*). Similarly, more eggs were obtained from female coccinellids fed on artificial diets. However, the larval predators showed slower rate of growth resulting in lighter pupae and smaller size adults when compared to those fed on aphids. Diet formulation seemed quite critical in determining diet acceptance especially by larvae. While the adult predators can easily accept dry, powdered formulation but the larvae will only feed on slightly moist formulation. Egg hatchability was high (70-80%) for both diet regimes. The inclusion of chicken liver is critical as it is a good source of protein which is vital for egg production. Research is now underway to encapsulate the liquid form of the diet using paraffin-wax combination.

20-004

THE INFLUENCE OF DIETARY PROTEIN HYDROLYSATES ON THE REPRODUCTION OF CERTAIN CHRYSOPIDS AND COCCINELLIDS

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Protein hydrolysates of yeasts (produced by autolysis and/or by adding proteolytic enzymes) either mixed or provided separately with sugars form suitable adult diets for inducing good egg production in many insects that feed on honeydews, nectars, or pollens. Partially hydrolyzed protein is superior to both unhydrolyzed and completely hydrolyzed proteins. For *Chrysoperla* spp. (*carnea* complex), a ratio of one part protein hydrolysate to two parts sugar is effective, but for predaceous species, e.g. *Chrysopa* spp. (*nigricornis*), *Harmonia* spp., *Olla* sp. and overwintered *Hippodamia convergens*, this ratio should be at least one to one. Predaceous adult diets should also contain cholesterol (egg yolk) ascorbic acid and tocopherol. Water is provided separately.

When protein hydrolysates of lactalbumin and/or casein are used, a ratio of one to one should be followed. B-complex vitamins must be added (which can be provided with yeast powder) along with egg yolk, ascorbic acid, and tocopherol. If casein hydrolysate is used without other proteins, cystine should be added.

20-006

PREDACEOUS PENTATOMIDS ON ARTIFICIAL NUTRITIONAL DIETS: ENZYMOLOGICAL ASPECTS

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To our opinion, difficulties of predaceous *Pentatomidae* rearing *in vitro* are conditioned by specific peculiarities of feeding and digestion mechanisms of this group of insects. To reveal the above mentioned peculiarities we study localization, composition and properties of the main digestive enzymes of *Podisus maculiventris* (Say) and *Perillus bioculatus* (Fabr.) (Hemiptera: *Pentatomidae*) larvae. Activity of proteolytic and amylolytic enzymes under the absence of that of lipase was found in salivary glands of both species. Conclusion was made on the importance of non - midgut digestion of protein and carbohydrate food components as an unlikely mechanism of non - midgut digestion of fat by predaceous *Pentatomidae* larvae. It was established that digestive proteases of *P. maculiventris* and *P. bioculatus* as that of the majority of other bugs, are represented mainly by cathepsin-B-like enzymes, which differs them from many other insects using trypsin-like proteases for digestion of proteins. It results in the necessity of careful selection of protein components for artificial nutritional diets for predaceous *Pentatomidae* according to hydrolysing ability of cathepsin-B-like enzymes from their alimentary canal.

20-005

EXTRA-CELLULAR MATRICES: WHAT ROLE FOR ENTOMOPHAGOUS INSECTS IN ARTIFICIAL DIETS?

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Most predators and probably parasites ingest their prey (or hosts) as liquids. However, the tissues of the prey are composed of cells that are held together by a complex matrix composed of collagen, elastin, glycosaminoglycans (GAGs), including hyaluronic acid, chondroitin sulfate and heparin as well as cell membrane-connected proteins. This complex, which gives tissues structure and stability, is a barrier to diffusion of digestive enzymes; and it may also be a source of nutrients to the entomophage. Some predators have special digestive enzymes, such as elastases, that attack some components of the inter-cellular matrix, helping to liquefy the prey's contents. Questions are raised here as to the possibility that such a matrix is desirable in artificial diets for predators.

20-007

IN VITRO REARING OF *EUCELATORIA BRYANI* SAB. AND *EXORISTA LARVARUM* (L.) (DIPTERA: TACHINIDAE)

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Eucelatoria bryani Sab. and *Exorista larvarum* (L.) are two parasitoids that attack important pest in agriculture. *In vitro* rearing system of *E. bryani* was made more economical reducing free aminoacids by half and adding yeast powder at varying concentrations. Tissue culture media added with insect components were used in order to improve the flies size.

E. larvarum was reared on meridic diets based on two tissue culture media (TNM-FH, SCHNEIDER'S), egg yolk, bovine serum, wheat germ and yeast without insect components. An average of about 20% of the eggs regularly failed to hatch. The adult yield reached was around 50% and the puparia average weight values were higher than 60 mg.

20-008

POSSIBILITIES OF MASS PRODUCTION OF THE PARASITOID *EXORISTA LARVARUM* (L.) (DIPTERA: TACHINIDAE) ON OLIGIDIC DIETS

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Attempts have been made by our research group to rear *in vitro* 4 tachinid species. Successful results, such as to permit the mass production in artificial media, however, have so far been obtained only for *Exorista larvarum*, a gregarious larval parasitoid of many Lepidoptera. Ease of rearing *E. larvarum in vitro* may be related to the simple relationship between this parasitoid, which is an idiobiont, and its host. Since larvae induce primary integumental respiratory funnels, they may display similar behaviour in the host and in the gelled diet.

Testing was mainly carried out using oligidic diets. The complete development of *E. larvarum* was obtained on different media, the simplest one containing distilled water, yeast extract, chicken egg yolk and saccharose. Rearing techniques were progressively simplified. In particular, plastic multi-well plates, that were originally used as rearing containers, were replaced with glass Petri dishes, in which the larvae can be cultured gregariously, instead of separately.

In the most suitable media, the percentage yields of adults, based on the number of eggs placed on the diet, were similar to those usually obtained in the factitious host *Galleria mellonella* L. (≈50%). In the laboratory, adults obtained *in vitro* mated, parasitized *G. mellonella* larvae, and produced a normal second generation.

When the above diet was integrated with 5% homogenate of *G. mellonella* larvae, the puparia weighed considerably more than those usually obtained *in vivo*. For this reason, as well as to secure a sort of "biochemical bridge" between the two symbionts, we think that it may be better not to completely eliminate host material from the artificial medium. At the moment, however, the host has to be necessarily reared in order to obtain *E. larvarum* macrotype eggs to be transferred onto the diet. In fact, oviposition by the adult onto artificial substrates has not yet been obtained.

20-010

IN VITRO REARING OF HOUSE FLY PARASITOIDS (HYMENOPTERA: PTEROMALIDAE)

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In vitro rearing of pteromalid wasps (*Muscidifurax zaraptor*, *Spalangia endius*, *Nasonia vitripennis*), pupal ectoparasitoids of *Musca domestica* and other filth flies, was successful utilizing several diets, with or without insect components.

The three species developed from egg to the adult on media containing insect material, extracted from pupae of either the natural host or the non-host species, *Heliothis virescens*.

M. zaraptor and *S. endius* attained the adult stage also on diets containing an amount of pupal extract as low as 5% (v/v). Further, *M. zaraptor* completed their development on diets devoid of insect components, though at lower rates.

The adult parasitoids reared *in vitro* successfully oviposited on host pupae, producing viable offspring.

While in nature *M. zaraptor* is a solitary parasitoid, several larvae successfully developed and reached the adult stage *in vitro* in the same well.

The results suggest that, at least for *M. zaraptor*, no special "host factors" are necessary to support larval growth.

20-009

IN VITRO REARING OF PARASITOIDS

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In vitro rearing of a parasitoid wasp and a fly by artificial diets is reviewed for research on physiology of their growth and development. A gregarious larval parasitoid wasp, *Cotesia kariyai*, was reared *in vitro* by insect tissue culture media containing silkworm haemolymph (BMH). After 4 days culture, the eggs hatched to 1st instar larvae released with hundreds of teratocytes. Both the larvae and teratocytes greatly increased their volume and maintained for at least 2 weeks.

A tachinid parasitoid fly, *Exorista japonica*, can be reared *in vitro* from eggs to adults by artificial diets. Added absorbent cotton immersed in each culture well caused to increase formation of the respiratory funnels in the 1st instar larvae soon after hatching. BMH with the cotton was the best diet for obtaining about 20% of adult flies.

20-011

IN VITRO REARING OF *APHIDIUS ERVI* HALIDAY (HYMENOPTERA, BRACONIDAE)M. C. Digilio, F. Pennacchio¹, P. Fanti¹, E. TremblayDipartimento di Entomologia e Zoologia Agraria, Università di Napoli "Federico II", Portici (NA), Italy - ¹Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, Potenza, Italy

Aphidius ervi Haliday is an endophagous larval parasitoid of aphids, with a rather broad host range, which includes several species of economic importance. *In vitro* rearing of this parasitoid species has been attempted. A simple artificial diet devoid of insect material has been developed by adding to the cell culture medium IPL-41 the following ingredients: fetal bovine serum, bovine albumin and chicken egg yolk. The addition of this latter component stimulated the larval growth from mature embryo to last instar. However, while larval size and developmental time of 1st instars are similar to those registered *in vivo*, 2nd instars and mature larvae showed a reduced growth. Based on *in vivo* physiological studies (Pennacchio *et al.*, 1995, Arch. Insect Biochem. Physiol., 30, 351-367), selected host-derived components are being tested *in vitro* to assess their nutritional value. The oviposition behaviour has also been considered. Physical cues play a fundamental role in eliciting parasitoid oviposition. In fact, a specific artificial colour applied to aphid dummies can stimulate an ovipositional response in 95% of tested females. This information is being used to develop a technical device to obtain parasitoid oviposition on artificial substrates.

20-012

GROWTH AND DEVELOPMENT OF THE ENDOPARASITOID *MICROPLITIS CROCEIPES* (BRACONIDAE) IN VITRO AND ON FACTITIOUS HOSTS

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Newly laid eggs of *Microplitis croceipes* must be exposed to components normally encountered in host hemolymph to initiate development. This requirement was met by incubating pre-germ band eggs of *M. croceipes* in IPL-52B, Grace's, and ExCell 400 media preconditioned for 24 hrs with five lepidopteran cell lines derived from fat body (IPLB-LdFB), imaginal wing discs (IAL-TND1, IAL-SFD1), or pupal ovaries (BCIRL-HZ-AMN1, BCIRL-HV). Egg development was dependent upon the composition of the medium, and the species and tissue type of the cell line source. The two imaginal disc cell lines and the fat body cell line were most effective in promoting germ band formation; whereas, the fat body cell line and the *T. Ni* imaginal wing disc-derived line were the most effective in promoting hatch. Because eggs developed only to the first instar in vitro, we examined the potential of rearing the parasitoid on six atypical hosts that are less expensive to rear than the natural host. Acceptance of hosts for oviposition by *M. croceipes* was accomplished by treating the larvae with frass plus hemolymph from *H. zea* larvae. Only *Galleria mellonella* and *Spodoptera frugiperda* allowed development of the parasitoid to the adult stage; however, the size of the resultant adult parasitoids was smaller and the numbers produced were lower than those parasitoids reared on *H. zea*. Efforts to reduce encapsulation of the parasitoid by treating the host larvae with gamma radiation, extreme temperatures, and phenylthiourea and supplementing the host's diet to enhance the nutritional suitability of the host failed to improve emergence of adult parasitoids.

20-014

IN VITRO REARING OF *CATOLACCUS GRANDIS* (HYMENOPTERA: PTEROMALIDAE) AN ECTOPARASITOID OF THE BOLL WEEVIL (COLEOPTERA: CURCULIONIDAE)

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The pteromalid *Catolaccus grandis* (Burks), an obligate ectoparasitoid of the boll weevil (*Anthonomus grandis* Boheman), successfully completed development on three original meridic diets. The amino acid content of diet α was established from HPLC analysis of the hemolymph of healthy third instar boll weevil larvae. The amino acid contents of diets β and γ was established from similar analysis of haemolymph of boll weevil larvae envenomed by *C. grandis* adult females. These diets were superior to a previously reported meridic diet for *C. grandis*. Pupal weight and fecundity of females reared in the three meridic diets were lower than for females reared in vivo, but, higher than for females reared in a previously developed diet.

The biological parameters of first, second, fifth, and tenth generations of *C. grandis* reared in the γ diet were evaluated. In vitro rearing of *C. grandis* for 5 successive generations did not affect the fecundity of the adult females. However, females reared during 10 generations in artificial diet showed significantly lower fecundity than all other generations tested. Pupal weight was not significantly affected by successive generations of in vitro rearing.

20-013

COMPARATIVE HOST-SEEKING BEHAVIOR, FECUNDITY, AND LONGEVITY OF ARTIFICIALLY-REARED VERSUS HOST-REARED *DIAPETIMORPHA INTROITA* (CRESSON) (HYMENOPTERA: ICHNEUMONIDAE) WASPS

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Diapetimorpha introita (Cresson) (Hymenoptera: Ichneumonidae) is a native ectoparasitoid of *Spodoptera* spp. (Lepidoptera: Noctuidae). An artificial culture media (no insect components present) has been prepared based upon proximate analyses of pupae of the host species. The media is presented in an artificial unit intended to simulate the host pupa. *D. introita* larvae feed through an artificial membrane and develop completely on the artificial media diet.

In this study, wasps developing from the artificial diet and from host pupae were compared for their developmental time, weight, fecundity, and longevity, and their ability to seek and to parasitize hosts.

The artificial media successfully produced *D. introita* wasps in the absence of any insect host components. These diet-reared wasps demonstrated a propensity to search for and attack natural host in field cage trials. Longevity of the diet-reared wasps was comparable with the longevity of wasps reared on natural hosts.

20-015

THE SYNERGISTS FOR IMPROVING THE EFFICIENCY OF IN VITRO REARING EGG PARASITOIDS

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The method of in vitro rearing egg parasitoids has been studied by the Guangdong Entomological Institute for 20 years. More than 16 species of *Trichogramma* and *Anastatus japonicus* Ashmead now can be reared in vitro, but there are still some problems which should be solved, such as the low and unstable parasitism of "artificial host-eggs". It reduces the efficiency of producing egg parasitoids with in vitro rearing method. In recent 3 years, we found some synergists which improved the parasitizing efficiency of *Trichogramma dendrolimi*, *T. confusum*, *T. evanescens*, *T. brassicae*, *T. pretiosum* and *Anastatus japonicus* significantly on the "artificial host-eggs". They are solution of polyvinyl alcohol, gelatin, white latex, agar, cassava powder, rice flour and starch. Among them the most effective synergist is the polyvinyl alcohol.

20-016

LYOPHILIZED ARTIFICIAL MEDIA FOR *IN VITRO* REARING OF *TRICHOGRAMMA* SPP. (HYMENOPTERA: TRICHOGRAMMATIDAE)S. Grenier¹, S.C. Han², L. Chapelle¹, W.H. Liu²¹ Laboratoire de Biologie Appliquée, LA INRA, INSA Lyon Villeurbanne, France - ² Guangdong Entomological Institute, Guangzhou, China.

To make easier the rearing in artificial media for the oophagous parasitoids of the genus *Trichogramma*, it is possible to use lyophilized media. These media can be prepared when biological material such as insect hemolymph is available, and stored for one year before use.

The performance of rehydrated lyophilized media (containing pupal hemolymph of *Philosamia cynthia* or *Mamestra brassicae*) were tested with the artificial egg card systems for rearing 2 strains of *Trichogramma dendrolimi* from China (TdC) or Italy (TdI) and *Trichogramma brassicae* (Tb). All experiments had concerned 5560 artificial eggs.

Percentages of parasitization, pupation and emergence were similar or higher with rehydrated lyophilized media kept for one year, than with fresh media. Lyophilized media, centrifuged after rehydration could induce a higher egg laying rate than non centrifuged ones: little modifications of the balance between free and total amino acids could be involved. As far as centrifugation effects were concerned, performances varied according to the result parameters considered, the medium used and the strain of *Trichogramma* tested. With TdC, non centrifuged media were a little better than centrifuged ones; with TdI no significant differences were observed and with Tb, centrifuged media were better than non centrifuged ones.

Lyophilization, which does not alter the performances of the media, is a good process to keep artificial media for a long time.

20-018

DEVELOPMENT OF AN EFFECTIVE PROGRAM FOR AUGMENTATION OF *TRICHOGRAMMA* AGAINST CODLING MOTH IN CALIFORNIA

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Trichogramma platneri is an indigenous egg parasitoid of the codling moth, *Cydia pomonella*, in California and can achieve up to 60% parasitism of codling moth eggs in unsprayed orchards later in the growing season. As part of a research program to reduce reliance on insecticide treatment of codling moth, we are developing an inundative release program for *T. platneri* in combination with mating disruption in pear orchards.

The two main questions that we have investigated are (1) how does release rate affect the level of suppression of codling moth damage, and (2) how is the impact of a constant release rate affected by the number of release points in an orchard? Release rate was investigated using treatments of 0 (control), 123,500, 247,000, 494,000 and 988,000 *T. platneri* ha⁻¹ wk⁻¹ in a replicated block design of 0.2ha plots. The number of release points in an orchard was similarly investigated using a constant release rate of 494,000 parasitoids ha⁻¹ wk⁻¹, with releases in every tree, every second or every fourth tree and a non-release control. Parasitism was monitored using sentinel eggs and damage was estimated by visual inspection of fruit in the center of each plot. The results confirm the absence of parasitism from wild *Trichogramma* populations in control plots and significant reductions of fruit damage in *Trichogramma* release plots. The optimal release rate was determined to be about 400,000 parasitoids ha⁻¹ wk⁻¹ and the extent of reduction in fruit damage was directly related to the number of release points in a plot.

20-017

IN VITRO REARING OF *TRICHOGRAMMA MINUTUM* RILEY (HYMENOPTERA: TRICHOGRAMMATIDAE) FOR MULTIPLE GENERATIONS AND OTHER ADVANCES IN THE DEVELOPMENT OF AN *IN VITRO* REARING SYSTEMD. A. Nordlund, Z. Wu¹, S. M. Greenberg¹USDA, ARS, Biological Control of Pests Research Unit, Weslaco, Texas, USA - ¹ Texas A&M University Agricultural Experiment Station, Weslaco, Texas, USA

Trichogramma minutum Riley were reared on an artificial diet for 10 generation. Several quality control criteria; including adult emergence rate, development time, adult size and sex ratio; were monitored. In comparisons with *T. minutum* reared on *Helicoverpa zea* (Boddie) eggs, the values for the quality control criteria of *in vitro* reared insects was generally quite good, often better than those for *in vivo* reared insects.

Though the diet used for these studies may not be suitable for use in commercial mass rearing (development of a more chemically defined diet is in progress) of these parasitoids, it does facilitate the development of an automated mass rearing system that could be used in a commercial setting. Progress in the development of such a system, including techniques for collecting *Trichogramma* eggs, rearing to the adult stage, field release and necessary equipment will be discussed.

20-019

MATHEMATICAL MODELLING ON THE BIOLOGICAL ESTIMATES OF *TRICHOGRAMMA* SPP. AGAINST *HELICOVERPA ARMIGERA* (HB.) IN COTTON ECOSYSTEM (HYMENOPTERA: TRICHOGRAMMATIDAE - LEPIDOPTERA: NOCTUIDAE)N. Muthukrishnan, M.S. Venugopal R. Janarthanan C. Kailasam¹ and A. Sundaram¹

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Simple correlation and regression techniques relating rates of parasitism to host densities do not contribute much to the understanding of host-parasitoid interaction in the field. A more feasible approach is the development of simulation model based on behavioural parameters. Data were generated in cotton field on the per cent parasitisation of *Helicoverpa armigera* eggs (artificially seeded) by *Trichogramma* spp. (*T. brasiliensis*, *T. chilonis*, *T. japonicum* and *T. pretiosum*) in three doses (1.0, 1.5 and 2.0 lakhs/ha), at different distances (2m to 50m) from the point of release and in eight directions during summer and winter of 1993 to 1994 seasons. The data were processed suitably by non-linear bounded exponential model in order to predict radius of influence, per cent parasitisation, optimum spacing between release points and benefit cost ratio for the released and extrapolated doses. The non-linear minimization algorithm by Marquardt-Lavenberg was used on a PC AT 286 computer to determine the parameters of the model. Totally eight models were worked out, a model for each *Trichogramma* spp. and season. All the eight models gave highly significant coefficient of determination (R²). Model predicted parasitisation by *Trichogramma* spp. (up to 10%) within the radius of 44.5 to 60.8m from the point of release in winter and 15.3 to 34.9 m in summer season. Parasitoid species and release densities did not influence the dispersal. Predicted order of superiority with reference to per cent parasitisation was *T. chilonis* > *T. brasiliensis* > *T. pretiosum* > *T. japonicum*. Optimum spacing between release points and benefit cost ratio estimated were 30.0 to 62.0m and 35.0 to 54.1 for winter season. This was 12.0 to 38.0m and 3.2 to 34.0 in summer. Increase in optimum spacing and decrease in benefit cost ratio were noted due to increase in release densities.

20-020

SUCCESSFUL COMMERCIAL DEVELOPMENT OF AN ENDEMIC *TRICHOGRAMMA* STRAIN AGAINST A TORTRICID LEAFROLLER IN GRAPEVINES.

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Inundative release of *Trichogramma* depends not only on effective parasitism of target pests in the field, but also on the ability to mass rear the selected species economically. Therefore new release programs often rely on commercially available material. However better results may be obtained if new strains are selected based on their field performance on specific target pests on the target crop after screening for acceptance of the mass-rearing host. This approach was utilised to develop an effective *Trichogramma* strain for commercial use against the Tortricid leafroller *Epiphyas postvittana* (Walker) in grapes in Australia. Four candidate species of *Trichogramma* were collected from *E. postvittana* egg masses in vineyards and assessed in the laboratory for their suitability for commercial use. This led to the selection of a strain of *Trichogramma* (*Trichogrammanza*) *carverae* which was reared on *Sitotroga cerealella* and tested in small plot trials within commercial vineyards using sentinel cards to assess potential release rates, spacing, release methods and dispersal patterns within the vineyard. These trials showed that over 75% of the egg masses in the vineyard could be parasitised by *T. carverae* at release rates of 70,000/ha with as few as 30 points per ha. Monitoring systems for *E. postvittana* were developed to help determine effective timing of releases. This study illustrates a relatively quick and inexpensive approach to the development of new strains of *Trichogramma* for commercial use that could be adapted for other agricultural systems.

20-022

THE USE OF ITS-2 DNA SEQUENCES FOR THE IDENTIFICATION OF *TRICHOGRAMMA* SPP.Richard Stouthamer, Frenk van Kan, Isabel Silva and John D. Pinto¹Department of Entomology, Wageningen Agricultural University, Wageningen, The Netherlands, ¹Department of Entomology, University of California, Riverside, CA USA

The small size and the few morphologically distinct structures in the genus *Trichogramma* has made the identification of the members of this genus difficult. At the same time *Trichogramma* wasps are mass reared and used extensively for the biological control of many lepidopteran pests. During mass rearing contamination of locally abundant *Trichogramma* species has happened, which led in some cases to the replacement of the intended species. Such contaminations have resulted in failed biological control because species specialized in the herbaceous habitat were released to control eggs in orchards. Clearly there is a need for a simplified identification system that will allow non-specialists to identify *Trichogramma* wasps. Here we will present a system based on the DNA sequence of *Trichogramma* species that will help anybody with access to a PCR-machine in the identification of specimen. We eventually hope to broaden the applicability of this system by producing an identification key based on the DNA sequence of the ITS-2 and other ribosomal genes.

20-021

TRICHOGRAMMA EVANESCENS; A BIOCONTROL AGENT AGAINST THE SUGAR-CANE BORER, CHILO AGAMEMNON (HYMINOPTERA: TRICHOGRAMMATIDAE-LEPIDOPTERA: PYRALIDAE)

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Plant Protection Research Institute, Dokki, Giza,

Trichogramma evanescens is reared in the laboratory on the eggs of the Angoumois grain moth, *Sitotroga cerealella*. Methods of rearing are described. The parasitoid has been released in sugar-cane fields against the sugar-cane borer, *Chilo agamemnon* since 1987. The treated area increased gradually from 20 feddan in 1987 to 11,000 feddan in 1994. The parasitoid is released only once, early in the season, at the rate of 20,000/feddan (48,000/ha). 50-79.3% reduction in rate of infestation is achieved at the end of the season. Total cost of rearing and releasing *Trichogramma* is almost three Egyptian pounds/feddan.

20-023

GREGARIOUSNESS IN EGG PARASITIDS: TOLERANCE OR LARVAL MOBILITY?

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Insect parasitoids can be either solitary or gregarious in a given host. In solitary species of Mymaridae, the elimination of supernumerary individuals is done mostly through physical competition between the mymariform larvae. We propose that, in the *Anaphes* genus (Hym.: Mymaridae), gregariousness is a secondary character that has evolved from solitary species. The current models explaining the apparition of gregariousness have proposed a "tolerance gene" to explain the ability of several larvae to develop together in a host. This "tolerance gene" could in fact be a reduction in the mobility in the mymariform larvae that remain however able to fight. Such a model can explain the apparition and persistence of gregarious species competing with closely related solitary species.

20-024

PARASITISM OF *CLAVIGRALLA GIBBOSA* SPINOLA (HEMIPTERA: COREIDAE) EGGS BY *GRYON* SP. NR. *GNIDUS* NIXON (HYMENOPTERA: SCELIONIDAE) IN INDIA

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Clavigralla gibbosa Spinola (Hemiptera: Coreidae) is an important pest of pigeonpea (*Cajanus cajan* Millsp.) in India. *C. gibbosa* females lay eggs in clusters on leaves and pods of *C. cajan*, and nymphs and adults feed on the developing seeds. Field collections over two years showed that the size of egg clusters is larger and more variable in the field (2-62 eggs) than has been reported from laboratory studies (3-33 eggs). The mean number of eggs per field-collected cluster was 18.3 compared to 6.1 in laboratory produced clusters. *Gryon* sp. nr. *gnidus* Nixon (Hymenoptera: Scelionidae) is a solitary egg parasitoid which commonly attacks *C. gibbosa* eggs on pigeonpea in India. Over the two years of this study, 13 to 68% of eggs were parasitized in each sampling period. Of the more than 40,000 eggs collected, *G. sp. nr. gnidus* parasitized 41.8%. The proportion of egg clusters parasitized varied from 22 to 100% and increased through the season. In 25 of 30 standard weeks sampled, more than 50% of egg clusters were parasitized, indicating the strong host finding ability of this species. The relationship between egg cluster size and parasitization showed a strong positive correlation; larger clusters were more frequently parasitized than smaller clusters. Less than 10% of egg clusters had all eggs parasitized by *G. sp. nr. gnidus*. The average size of egg clusters in which all eggs were parasitized was 15.2 eggs.

20-026

TAXONOMY OF TRICHOGRAMMATIDAE
J.D. Pinto (Sun City-United States)

ABSTRACT NOT RECEIVED

20-025

INFLUENCE OF VARIOUS EGG HOSTS ON THE BIOLOGY OF TRICHOGRAMMA CHILONIS ISHII (HYMENOPTERA : TRICHOGRAMMATIDAE)

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Observations were made on some biological parameters of *T. chilonis* reared on the eggs of *Helicoverpa armigera* (Hb.), *Amsacta moorei* Butler* and *Corcyra cephalonica* Stainton. Amongst different hosts, *E. vittella* was preferred by the parasitoid both under choice and no-choice conditions. Per cent adult emergence was highest when the parasitoid was reared on *C. cephalonica*. Greater proportion of females in the parasitoid progeny emerged from the eggs of *A. moorei*. Parasitoids emerged from the eggs of *E. vittella* were more fecund and long lived. Interestingly, parasitoid progenies from larger eggs (of *A. moorei*) were less fecund and short lived necessitating further investigations.

20-027

GENETIC MAPPING OF TRICHOGRAMMA BRASSICAE GENOME WITH RAPD MARKERS

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Although biological control represents a successful pest control strategy, selective breeding programs have hardly begun for natural enemies, such as the parasitoid wasp *Trichogramma*. Since genetic maps provide a powerful tool for accelerating breeding through marker assisted selection, a genetic map of *Trichogramma brassicae* genome is under construction with RAPD markers. Later, location of the genes controlling fecundity and longevity will inform us about the feasibility of QTL (Quantitative Trait Loci) identifications of characters involved in the biological control efficiency.

Three F2 mapping populations of 100 individuals each were constructed. These populations are polymorphic for both DNA and biological traits. 192 10-bases random primers were screened for polymorphism between parental lines and homogeneity inside lines. A total of 116 RAPD fragments (i.e. markers) obtained from 74 primers were selected for mapping.

At present, segregation of a total of 60 RAPD markers was scored on the various mapping populations. As preliminary results show a good colinearity of the 3 genetic maps, and thus assess the reliability of the RAPD markers to construct a genetic map of *Trichogramma brassicae* genome, a composite map of 51 markers has been constructed. Four groups which putatively correspond to the 4 largest chromosomes out of 5 were identified. More complete genetic maps will be presented.

20-028

EFFECT OF DENSITY RELEASE OF *USCANA SENEX* GRESE (HYMENOPTERA: TRICHOGRAMMATIDAE) ON THE PEA WEEVIL EGGS PARASITIZATIONM. Gerding¹ and L. Hormazabal²¹Centro Regional de Investigación Quilamapu-INIA, Chillán, Chile.²Facultad de Agronomía, Universidad de Concepción, Chillán, Chile

An experiment was conducted to evaluate the effect of four different release densities of *Uscana senex* on the parasitism ability of *Bruchus pisorum* eggs. Colonization and pea seed damage reduction were evaluated. The total release treatment were 1.92, 3.84, 7.68 and 15.2 millions of *U. senex* ha⁻¹. Each treatment considered two release per week from beginning of flowering to 100 % of full pods, unto complete the total of individual per treatment. The rate of parasitism obtained in the treatments with parasitoids ranged between 49.8 % and 82.1%.

U. senex exhibited an important dispersion ability and the eggs parasitism rate decreased as the distance from the focus of release increase. Seed damage was reduced in 70% with the highest release density as compared with the check

20-030

USE OF TRICHOGRAMMA IN *RHYACIONIA BOULIANA* CONTROL IN CHILEMarcos Gerding¹, Ernesto Cisternas² and Cecilia Céspedes¹¹Centro Regional de Investigaciones Quilamapu, Chillán Chile.²Centro Regional de Investigaciones Remehue, Osorno Chile

Since 1985, *Rhyacionia bouliana* (Lepidoptera: Tortricidae) has been the main pest of *Pinus radiata* forest in Chile. Its incidence has been increasing every year, with a significant economic injury, riching up to 50% of timber production. The biological control program has been oriented to introduce *Orgilus obscurator* (Hymenoptera: Braconidae) but, its mass rearing is highly time consuming. The use of *Trichogramma* spp., as biological control complement, could be a useful alternative for the pine growers. Recently, a native *Trichogramma* from *R. bouliana* eggs was collected in the central-south area of Chile. Under laboratory conditions this parasitoid was mass reared and its parasitic ability compared with *T. dendrolimi* and *T. telengai* strains, introduced from Bulgaria. The results were favorable to the native trichogramma. In the last season, the field release studies, about density and opportunity, has been done only on this native egg parasitoid.

20-029

BIOLOGICAL CONTROL OF THE DIAMONDBACK MOTH BY TRICHOGRAMMA CHILONIS

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We have been investigating the possibility of a biological control program against the diamondback moth (DBM), *Plutella xylostella*, using *Trichogramma chilonis*. Here we would clarify the properties of *T. chilonis* and examine how to utilize them. *T. chilonis* females which developed in DBM eggs showed a lower fecundity than those developed in *Ephestia kuehniella* for mass rearing host. *T. chilonis* females which developed in DBM egg did not have a markedly higher reproductive capacity. Under conditions of high temperature, a smaller percentage of emergence of the progeny would be expected. These results indicate that the inundative method would be better than inoculative release for control of DBM using *T. chilonis* developed in alternative hosts in the laboratory in warmer temperate zones.

To simulate the population dynamics of the system of the DBM and *T. chilonis*, it was made a simple simulation model described as the Leslie Matrix model based on following behavior. *T. chilonis* would oviposit in a parasitized host after release under some conditions (e.g., higher parasitoid densities). *T. chilonis* could attack DBM eggs of all ages, but the intermediate age host is most parasitized in all age-hosts. In response to a contaminated area where DBM scales and secretions present, a *T. chilonis* female exhibits a response involving walking at a reduced speed. DBM scales or secretions elicited the oviposition behavior of *T. chilonis* females. From functional response of *T. chilonis* to DBM eggs, rate of attacking increased rapidly as the DBM egg density increased, but the curve levelled off at higher host densities. The model indicated that an optimal time and optimal density of releasing parasitoids exist in the introduction programme of *T. chilonis*.

20-031

HOST LOCATION BY PARASITOID: ASSESSMENT OF PROFITABILITY

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Like all foraging animals, insect parasitoids have to cope with temporal and spatial variability in resources. Parasitoids make flexible use of chemical information from their host and its food to optimize life time host encounter rate. In contrast to what is assumed in many foraging models, foraging parasitoids are not omniscient but need to learn through experience where hosts are and which (micro)habitats are the most profitable. Chemical host and plant cues can give information on the presence and even on the relative density of hosts, and the reliability of this information is enhanced through learning. It is known that experience can lead to induced preferences for important foraging cues. It is less known that parasitoids also decide to ignore experienced variation and that they generalize learned information. I will present data on the assessment of habitat profitability e.g. showing that the expression of preference is influenced by the informational state of the parasitoid, i.e. by whether the animal has 'complete' or 'incomplete' information on the profitability of different microhabitats.

20-032

THE ROLE OF PLANT CHEMICALS IN HOST SELECTION BY PARASITIC INSECTS.

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Parasitic insects use several chemicals of host food plant origin as cues of presence of hosts. The functions of such cues differ according to their volatility: parasitoids can detect volatile cues at a distance from a host food plant whereas they can detect nonvolatile cues through contact chemoreception. In addition, plants produce these chemicals either constitutively or in response to herbivore damage. The damage by host feeding may induce a plant to produce specific compounds. Such host-induced plant chemicals seem to be more reliable cues for the parasitoids on the presence of host on the plant than constitutively produced chemicals. We will report on the role of such plant chemicals in the host selection by parasitic wasps in the following two systems.

(1) Crucifer plants - *Pieris rapae* larvae - *Cotesia glomerata*

(2) Corn plants - *Pseudaletia separata* larvae - *Cotesia kariyai*

20-034

PLANT-MEDIATED SUITABILITY: DOES HERBIVORE PERFORMANCE DETERMINE PARASITOID FITNESS? B. Benrey. Center for Ecology, Universidad Nacional Autónoma de México (UNAM).

A growing number of studies suggest that plants can dramatically influence the interactions between herbivorous insects that feed on them and the natural enemies of these herbivores. Features of plants, such as allelochemistry, nutrients, and morphology, can affect herbivore-enemy interactions by influencing the enemies either directly or indirectly.

Systems in which wild and cultivated species coexist provide ideal models to study interactions among the three trophic levels. Crop plants and ornamentals which have been the subject of artificial selection may have altered allelochemistry and nutrient content. Insects that feed on these plants, as well as the natural enemies that attack these insects have been forced to adapt to this plant variability. I will present the results from studies conducted on two systems; one involving the herbivore *Pieris rapae* (Lep: Pieridae) and its parasitoid *Cotesia glomerata* (Hym: Braconidae) and the other involving the seed eating beetle *Zabrotes subfasciatus* (Col: Bruchidae) which is parasitized by *Stenocorse bruchivora* (Hym: Braconidae). In these studies we examined the influence of plant species on the performance and host location behavior of parasitoids that were offered hosts on cultivated and wild plant species. Results from these two studies show that overall, the plant species on which hosts perform best, also provide the most suitable resource for the parasitoids. It was found that, parasitoid fitness is increased by parasitizing the most vigorous hosts. I discuss how natural selection may have operated on parasitoids to locate plants with hosts in which their performance is maximized.

20-033

HERBIVORE-INDUCED EMISSIONS OF PARASITOID ATTRACTANTS BY PLANTS

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A multitude of studies on tritrophic level interactions have shown that plants can promote the presence of natural enemies of herbivores. Particularly, the phenomenon that plants emit specific odors in response to herbivore attack has received considerable attention. One measurable consequence of the odor emissions is the attraction of parasitic wasps, which is suggested to be one of the functions of the plant-produced odor. A comparison of herbivores that employ different feeding strategies revealed considerable differences in the emissions that they induce plants to emit. Some insects may be able to avoid induction and therefore reduce the chances that they are found by their natural enemies. The additional consequences of herbivore-induced emissions of plant volatiles and strategies that herbivores may employ to avoid these consequences are discussed.

20-035

FORAGING FOR HOST AND FOOD RESOURCES: COMPARISONS, CONTRASTS, AND INTERACTING INFLUENCES

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Parasitic insect adults must locate food to meet their short-term nutritional needs as well as find hosts for reproductive purposes. A knowledge of how females deal with the often competing needs for these two vital resources is essential to understanding parasitoid foraging strategies. Studies show that foraging females need periodic nectar and/or pollen meals, and that availability and accessibility of food in a target area strongly affects their retention and host finding efficacy.

The use of olfactory and visual cues from plants, usually enhanced by learning, play important and sometimes interacting roles in a female parasitoid's search for food and hosts. Thus, the provision by plants of floral and extrafloral nectar along with associated foraging signals play a crucial role in the tritrophic interplay of plants, herbivores and parasitoids. This broader understanding of tritrophic level interactions that encompasses parasitoid food considerations can enhance our ability to design effective biological control strategies.

20-036

RELIABILITY VS. DETECTABILITY AS A FORAGING CONSTRAINT FOR FORAGING PARASITIDS AND PREDATORSMarcel Dicke

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Carnivorous arthropods such as predators and parasitoids that search for herbivores can potentially use a variety of chemical cues during various phases of the foraging process. In long-distance searching volatiles cues are the most important ones. Such cues may originate from the herbivore and from its food plant, but these two groups of volatiles differ in detectability and reliability.

The most important volatile cues that are used by carnivores, are herbivore-induced synomones that originate from the food plant of the herbivores. These chemicals, that are emitted by herbivore-damaged plants are highly detectable. In several cases they are highly reliable indicators of the herbivores that damage the plant, in others such reliability has not been recorded.

I will review knowledge about the reliability of herbivore-induced synomones. The major question is whether the reliability of herbivore-induced plant volatiles is idiosyncratic or can a pattern be found? How do parasitoids and predators deal with non-specific plant volatiles in finding the right herbivore species or instar.

20-038

THE ROLE OF VISUAL AND MECHANOSENSORY CUES IN HOST LOCATIONFelix L. Wäckers

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In addition to chemical stimuli, physical stimuli also play a crucial role in host searching by hymenopterous parasitoids. Parasitoids were found to use colour, shape, movement and mechanosensory cues (vibrations) for orientation. These physical stimuli may either stem from the host habitat, directly from the host, or (in the case of echolocation) from the parasitoid itself.

While some of the responses to visual and mechanosensory stimuli are innate, it could be demonstrated that parasitoids employ associative learning to incorporate additional physical stimuli into their search profile. These learned responses allow parasitoids to concentrate their search on those parts of the habitat that are most profitable in terms of host encounters.

Examples of visual and mechanosensory orientation will be presented and their relative significance in host finding will be discussed.

20-037

SOURCES OF INTER-INDIVIDUAL VARIABILITY OF ODOR LEARNING PERFORMANCES IN *LEPTOPILINA BOULARDI* (HYMENOPTERA: EUCOILIDAE), PARASITOID OF *DROSOPHILA*.R. Pérez-Maluf¹, L. Kaiser¹, E. Wajnberg²

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As in many parasitoid species, behaviors leading to host location by females *Leptopilina bouleardi* are triggered by the perception of chemicals cues, which depends on learning processes. Odors perceived during oviposition are memorized through associative learning, and consequently trigger oriented locomotion and ovipositor search of a substrate. Such changes in odor responses would increase the probability to locate a suitable host: associative learning is considered as a key process for the adaptation to varying host resources and for successful parasitism. Ability for associative learning varies greatly among individuals, as seen from laboratory studies conducted on *L. bouleardi*, so we started to search for the sources of such a variability. We developed a bioassay to quantify individual learning performances based on the observation of odor-conditioned probing response, characterized by several parameters. The relative importance of genetic versus environmental variability of these parameters was evaluated using isofemale lines. Because most variability was environmental, the influence of two non-genetic factors, mating and oviposition experience, was investigated. Possibilities to control better the variability of odor learning involved in host selection will be discussed.

20-039

LEAF VIBRATIONS AND NEAR FIELD AIR DISPLACEMENT IN A LEAFMINER-PARASITOID SYSTEMJ. Casas, S. Bacher¹, J. Tautz² and R. Meyhöfer¹

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The parasitoid *Sympiesis sericeicornis* (Hym. Eulophidae) changes its foraging behaviour on the mine when its host, the apple leafminer *Phyllonorycter malella* (Lep. Gracillariidae), initiates vibrations either by moving or escaping (Meyhöfer et al. *Physiol. Ent.* 19: 349-359, Meyhöfer et al. *submitted*). The host also reacts to the vibrations produced by the foraging parasitoid (Bacher et al. *Physiol. Ent.* *in press*). How specific or common these vibrations are in the environment of the leafminer was studied using Laser Vibrometry. Vibrations produced by simulated rain and wind were of a different nature to those produced by the parasitoid. Moving insects impart forces of varying intensities and duration to the leaf. It is therefore difficult to assess from such data how far leaf properties modify vibratory signals. Using impacts made by small metal balls, the ringing signature of the leaf was identified in a signal and it was established that a gradient in intensity and frequencies composition exists over the entirety of a leaf. Foraging parasitoids of endophytic hosts may use air displacement triggered by plant vibrations, in turn produced by moving hosts. Measurement using Laser Vibrometry coupled with Laser Anemometry around vibrating leaves confirmed that near field air displacement is a potential source of information to foraging parasitoids and hence deserves closer analysis.

20-040

EGG LIMITATION IN PARASITOIDS: EVIDENCE FROM FIELD STUDIES AND IMPLICATIONS FOR HOST SELECTION

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Recently-developed theory predicts that natural selection should favor a non-trivial risk of female parasitoids becoming egg-limited (i.e. depleting their egg supply). In this presentation, I address three questions related to this hypothesis. First, is there empirical support for egg limitation from field studies of parasitoids? Second, should we expect the risk of egg limitation to differ in natural versus agricultural populations of parasitoids? And third, what are the consequences of egg limitation for host selection?

The few studies aimed at uncovering the prevalence of egg limitation have all supported the hypothesis that egg limitation occurs in the field. Egg limitation apparently occurs under both natural and agricultural conditions, and it has been proposed that it may be more extreme under agricultural conditions, where host availability is likely to be high. Also, egg limitation occurs in pro-ovigenic species (where it is permanent), as well as in synovigenic species, where it may be temporary.

Parasitoids are predicted to be increasingly 'choosy' with respect to host selection as the risk of egg limitation increases. Evidence for increased choosiness under conditions of impending egg limitation is mounting, although some studies have failed to show such an effect.

20-042

STRATEGIES INVOLVED IN THE LOCATION OF HOSTS BY THE APHID PARASITOID *APHIDIUS ERVI* HALIDAY (HYMENOPTERA: BRACONIDAE: APHIDIINAE)

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The parasitoid *Aphidius ervi* Haliday uses both semiochemical and physical cues to locate its main host, the pea aphid *Acyrtosiphon pisum* (Harris). Recent wind tunnel studies have demonstrated the importance of plant volatiles in the initial, long range host finding responses, particularly volatiles induced by aphid feeding damage. However, the parasitoid can distinguish between plants damaged by host aphids and those damaged by the non-host *Aphis fabae* (Scop.). There is also evidence from wind tunnel bioassays that the host-induced plant volatiles are produced systemically throughout the plant. Volatile chemical entrainment and identification techniques are being used to isolate and identify the compounds involved in these responses. Previous experiences with hosts or host-related cues heighten the responses to host-induced plant volatiles and trigger other responses via conditioning and/or associative learning. Contact cues appear to be used in host recognition and acceptance and bioassays have indicated the presence of such cues in the host cuticle and cornicle secretion. Visual cues also play a role in host location and recognition, with colour being an important short range cue. The significance of these findings for the manipulation of adult parasitoid behaviour to enhance pest control and their implications for host selection and evolution within the Aphidiinae will be discussed.

20-041

THE INFLUENCE OF COMPETITION BETWEEN FORAGERS ON CLUTCH SIZE DECISIONS IN AN INSECT PARASITOID WITH SCRAMBLE LARVAL COMPETITION.

Marcel E. Visser

Netherlands Institute of Ecology, Heteren, The Netherlands

The effect of competition between ovipositing females on their clutch size decisions is studied in animals that lay their eggs in discrete units of larval food (hosts). In such species the effect of competition depends on the form of the larval competition within such units. In parasitoids insects, there might either be contest (solitary parasitoids) or scramble competition (gregarious parasitoids) between larvae within a host. For gregarious parasitoids, a decreasing clutch size with increasing competition between foragers is predicted. This prediction is tested in experiments using the parasitoid *Aphaereta minuta*. Parasitoids were either kept alone or in groups of 4 before the experiment, in which they were introduced singly in a patch containing unparasitized hosts. Animals kept together laid on average clutches of 0.74 eggs smaller than females kept alone (average clutch is 5.3), thereby confirming the prediction. Clutch size decreased with encounter number, which might be due to the adjustment of the female's estimate of the encounter rate with hosts. Finally, the results are compared with those reported for solitary parasitoids (that have scramble larval competition), for which it is predicted that the clutch size will increase with increasing levels of competition between females. In some species of solitary parasitoids, a clutch of 2 eggs is laid in a host under intra-specific competition between females. In other species the occurrence of self-superparasitism, which can be viewed as a clutch size adjustment, increases with increasing competition. Thus, as predicted, with increasing intra-specific competition between adult females, clutch size increases in solitary parasitoids and decreases in gregarious parasitoids.

20-043

HOST SELECTION STRATEGIES USED BY EGG PARASITOIDS

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Host eggs represent for parasitoids a small finite source, undergo major and often quick internal changes, probably lack internal defences, are immobile, and derive their protection from the preceding stage: the adult. Therefore egg parasitoids, as other idiobionts, use an array of cues from plants, host adult and host egg and have developed diverse and often unique host relationships. They are also important biocontrol agents. Several aspects are reviewed as follows.

Host associations focus on host habitat and plant, oviposition sites, host egg characteristics in several groups. Chemio-physical cues are examined in different biotaxonomic and tritrophic systems together with host preference and alternate hosts.

Efficiency and constraints are discussed with regard to discovery and exploitation of host and its patch, to impact and secondary sex-ratio of parasitoid, and to suitability of plant-host complex.

Novel associations show a success rate not significantly different from coevolved ones. These results cannot be explained without considering the equivalences in recognition factors between old and new potential hosts which make host shifting possible.

Basic studies of chemio-physical cues mediating host selection behavior should aim at defining predictability of specificity in order to test efficacy and safety of these parasitoids and transferring this to biocontrol programs.

20-044

THE EGGS OF THE PARASITOIDS.**J.-P. N  non and G. Boivin**

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The insect parasitoids evolved from species with different life histories and from several types of habitat. Different egg types evolved depending on the host and habitat exploited by these parasitoids. Among the constraints acting on the egg, the oviposition site (endo- ectoparasitoids or host found by mobile first-instar larva) and the length and type of ovipositor appear most important. Species from different groups, among the Hymenoptera but also the Coleoptera, Diptera and Trichoptera, facing the same situation may have evolved the same adaptations through convergence. The egg characteristics examined in species representative of these groups were the chorion, the envelopes and the vitelline content along with functional aspects such as the egg plasticity at oviposition and resistance to desiccation and host defenses.

20-046

A COMPARISON OF THE EXOCRINE GLANDS OF PARASITIC HYMENOPTERA AND THEIR BIOLOGICAL SIGNIFICANCE

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Hymenoptera harbor a variety of exocrine glands generally of ectodermal origin. These glands serve important functions in the biology of parasitic hymenoptera. Many of these glands are found in the head and are thought to be associated to feeding. Other glands are located in the abdomen and are assumed to be associated with oviposition. Although most exocrine glands are of ectodermal origin, for example, those associated with reproduction, such as Dufour's gland, the poison gland and glands associated to the lateral or common oviduct, follicular cells are of mesodermal origin, but can provide exocrine secretions. These and other glands to be discussed include the pygidial or Hagen's glands, and other glands in the abdomen suspected to produce exocrine secretions. The occurrence and morphology of these glands show considerable variation among the various taxa of parasitic hymenoptera. This variation and the function of these glands, when known, is discussed. However, this discussion will not include glands associated with the antennae, which will be included elsewhere.

20-045

MOUTHPART STRUCTURE AND FUNCTION IN INSECT PARASITOIDS, WITH PARTICULAR REFERENCE TO FEEDING**M.A.Jervis, F.S.Gilbert¹**

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The diversity of mouthpart structure among parasitoids is examined. The mouthparts of some Braconidae (so-called 'exodonts': Alysiinae, Dacninae) are modified for the purpose of escape from the host's puparium. In *Psilus* species (Diapriidae) the mandibles have a long, wide sweep, and are similarly employed. The majority of hymenopteran parasitoids are able, using the mouthparts, to lap up both wet honeydew and nectar from exposed nectaries. They can also easily ingest pollen grains. Perilampidae and Eucharitidae appear to use their digitate labrum to avoid ingesting pollen grains that often contaminate nectar. Adults of some Ichneumonidae (*Lapton*, *Agathophiona*, *Rynchophion*, *Gonolochus*, *Agathilla*, *Certonotus*) and Braconidae (species of *Bracon*, *Agathis*, *Cardiochiles* and *Chelonus*) have elongated suctorial mouthparts (labiomaxillary complex) (= apomorphic condition) that they use to consume concealed floral nectar.

The plesiomorphic condition for the mouthparts of Cyclorrhaphan Diptera (e.g. many Tachinidae) is a fleshy labellum with numerous pseudotracheae and their overlying channels. These structures are efficient in the ingestion of either honeydew or nectar (extrafloral or unconcealed floral), but can also easily serve in the removal and ingestion of pollen from the anthers of flowers. Members of a much wider range of families than in parasitoid Hymenoptera have elongated mouthparts (Acroceridae, Conopidae, Bombyliidae, Nemestrinidae, Phoridae, Pyrgotidae, Tachinidae); the labellum is reduced in these insects. Such mouthparts are associated with the exploitation of flowers that have deep corollae and sugar-rich nectar.

We propose areas for future research, including complementary behavioural studies.

20-047

ANTENNAL SENSORY AND SECRETORY STRUCTURES IN PARASITOID HYMENOPTERA**N. Isidoro, F. Bin, S. Colazza, S.B. Vinson¹**

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The antennae of Hymenoptera parasitoid, previously considered only as receivers of signals, are also emitters for intra- and interspecific communication. This revision describes sensilla and glands, suggesting their possible function and comparing them in different taxa. General features of all types of sensilla are revised following the conventional classification into aporous, uniporous and multiporous sensilla. For multiporous types, ultrastructural investigations have revealed special features which define some new sensilla, e.g. female gustatory sensilla with a specialized multiporous area innervated by very many sensory neurons. Secretory structures, belonging to class 1 or 3 gland type, may be present in one or both sexes. The gland release site may be apparent or inconspicuous and may be found on modified or unmodified antennomeres. In males the release site may be dorsal, ventral or lateral. In females it is generally dorsal with the exception of two groups which have ventral accessory glands associated with multiporous gustatory sensilla. Using morphological data, topography and behavioral observations, the role of some sensilla and glands is discussed. These structures are likely involved in both physical and chemical aspects of mate and host recognition.

20-048

Ovipositor structure and function in the parasitic Hymenoptera.

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The ovipositor and oviposition behaviour have been extremely important in the evolution of the Hymenoptera, and especially in the evolution of the parasitoid life history. In this chapter we review the functional anatomy of the hymenopteran external ovipositor system. The basic structure is derived from paired appendages of the 8th and 9th abdominal segments has been preserved throughout the order, and comprises an upper valve (2nd valvula) which is interlocked with a pair of lower valves (1st valvula) together with a pair of protective sheaths (3rd valvula). In nearly all non-aculeate Hymenoptera, the tubular canal formed between the upper and lower ovipositor valves serves for the passage of eggs and other secretions (e.g. venoms, lubricants, marking pheromones, feeding tube materials). The ovipositor valves contain living tissue including nerves, tracheae, and fat cells, but lack intrinsic musculature, and so substrate penetration and oviposition rely on muscles within the metasoma that attach to the bases of the ovipositor valves and associated sclerites. Some taxa have evolved telescopic ovipositor mechanisms that involve modified posterior metasomal segments, and some are able to steer their ovipositor tips to facilitate host location. The apices of the ovipositor valves are usually serrated, but little is known of 'drilling' mechanics. Passage of the egg along the egg canal is assisted by too-and-fro movements of the valves and is believed to result from ratchet-like action of the cuticular scales present on the egg canal wall. The microsculpture varies along the ovipositor within a species, and between species. The tip of the ovipositor and the ovipositor sheaths have sensillae. Mechanoreceptors are typically present on the 2nd valvifers; proprioceptors on 1st and sometimes 2nd valvulae are probably involved in detecting movements of the different pieces of the ovipositor during egg laying. Various sensilla morphotypes on the 3rd valvulae differ between species in number in accordance with use of 3rd valvulae in host examination. First and 2nd valvulae have various sensilla, including mechanoreceptors and contact chemoreceptors which are probably involved host discrimination and acceptance, and egg placement. Compared with others insect groups, sense organs on female genitalia in parasitic wasps are characterised by i) reduced size, ii) peculiar morphological types, iii) larger proportion of gustatory compared to mechanoreceptors. Some of the above features may be phylogenetically informative, whilst other characters are associated with particular types of hosts or host substrates, and exhibit adaptive convergence.

20-050

DIAPAUSE IN THE PREDACEOUS HETEROPTERAJ.R. Ruberson¹, T.J. Kring²¹University of Georgia, Tifton, GA, USA.²University of Arkansas, Fayetteville, AR, USA.

Heteropteran predators in the Temperate Zone must cope with predictable times of each year that are refractory to their development and reproduction: typically winter and portions of the spring and fall. Many species of heteropteran predators pass this period in a state of diapause. Most diapausing species do so as adults, although some diapause in nymphal stages and a few as eggs. Predaceous heteropterans usually overwinter individually in sheltered habitats, such as in leaf litter, under tree bark, or in winter grasses. Overwintering mortality of these species is not well known, but appears to be high for those species studied to date. Photoperiod serves as a primary cue for diapause induction, although its effect may be modified by temperature and, in at least one case, by the quality of prey. Sensitivity to diapause-inducing photoperiods occurs in either the nymph or the adult, or in some combination of both the nymphal and adult stages. When sensitivity occurs in the nymphal stages, it is restricted to the late instars for species studied thus far. Short photoperiods serve to maintain diapause in the predaceous Heteroptera, although low temperatures can interact with short photoperiods to maintain diapause; conversely, long photoperiods and high temperatures serve to maintain diapause. At least several species of predaceous Heteroptera feed while in diapause; thus prey availability may be important in selecting an overwintering site. Understanding diapause can be valuable for commercial production, where diapause can be an impediment to rearing and application, or a valuable tool for storing or stockpiling predators.

20-049

FUNCTIONAL MORPHOLOGY AND THE PRACTICAL USE OF PARASITOIDS IN BIOLOGICAL CONTROLJ. LaSalle, L.M. LeBeck¹International Institute of Entomology, London, UK - 1
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Biological control programmes are reviewed to assess the impact of studies on various aspects of functional morphology of parasitoids to the success of the projects. Examples are presented where problems in the implementation of successful biological control were solved through the previous knowledge or subsequent study of functional adaptations of parasitoids. Justification is provided for continuing applied studies of parasitoid morphology, behaviour, and biology. Recommendations are made for priority areas of future research activity.

20-051

MANAGEMENT OF PREDACEOUS HEMIPTERANS WITH SEMIOCHEMICALS: PRACTICE AND POTENTIAL

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Two ongoing research projects involving predaceous hemipterans will be discussed: 1) pheromone-mediated augmentation of the spined soldier bug (SSB), *Podisus maculiventris* (Say) (Pentatomidae), for suppression of the Colorado potato beetle (CPB) and, 2) the isolation and identification of the suspected sex pheromone of *Orius insidiosus* (Say) (Anthrenidae).

The SSB overwinters in the adult stage, and a synchronized, massive emergence occurs just prior to the bud burst of deciduous trees in the eastern U. S. The commercialized, male-produced pheromone of this predator (attractive to both sexes and nymphs) is being used to harvest wild adults in pheromone-baited traps to mass-produce and manipulate young predators for augmentative biological control. Results after the second year of a 3-year project indicate that the average potato plant defoliation by the CPB in May was 20% in the plots where the soldier bugs were released (ca. 5 nymphs/plant), and 44% where the bugs were not released (9 potato plots; 500 m²/plot; ca. 1036 plants/plot). The average potato plant in the release plots produced 454g of tubers whereas production in the control plots was 60g, an 87% yield reduction. For *Orius insidiosus*, Y-tube olfactometer bioassays showed that males are attracted to volatiles from virgin females. Suspected attractants were isolated by whole-body extraction, aeration, and microdissection. Three novel female-specific compounds have been identified: (2E,7)-octadienal, (2E,7)-octadienoic acid, and (2E,9)-decadienal. Further laboratory and field tests are planned to determine if these compounds are attractive to conspecifics.

20-052

PHYTOPHAGY IN PREDATORY HETEROPTERA AND ITS IMPLICATIONS FOR BIOLOGICAL CONTROL

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Many species of predatory Heteroptera are known to feed on plants, as well as prey (omnivory). Plant resources enhance the fitness of these predators not only in the absence of prey but also as supplements to a carnivorous diet. Studies show that plant species differ in their suitability for the development, survival, and reproduction of *Orius insidiosus*. On some plants, the oviposition preference of the females was correlated with the plants' suitability for the nymphs. A simple model shows that populations of plant-feeding predators and their prey are less stable when the omnivore feeds on suitable plant resources. Data on the response of omnivorous Heteroptera to plant diversity in the habitat suggest that the instability of systems with plant-feeding predators is local. Apparently, these omnivores respond in an opportunistic manner to changes in the availability of prey and plant resources. To enhance the efficacy of predatory bugs, yet prevent possible damage to crops, biological control programs need to consider the unique feeding habits of many promising heteropteran agents. Also, plant resources could be utilized as food, oviposition substrates, and delivery media in augmentative release programs.

20-054

COLONIZATION AND SEASONAL DYNAMICS OF *ORIOS INSIDIOSOS* IN NORTHERN CLIMATESA. Andow

ABSTRACT NOT RECEIVED

20-053

POPULATION DYNAMICS OF *ORIOS* AND ITS RELEVANCE TO BIOLOGICAL CONTROLYoshimi Hirose

Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

Although *Orius* species are known to be effective control agents of various pests, the population dynamics of *Orius* are examined in search of mechanisms involved in the biological control of thrips pests in particular. A population study of *Orius* spp. and the pest *Thrips palmi* on eggplant in home and truck gardens in Japan demonstrates that the more abundant the population of *T. palmi* per home garden, the more abundant the population of *Orius* spp. per home garden. There is suggestive evidence that this numerical response of *Orius* to the prey abundance is due to increased oviposition or reproduction rather than adult aggregation. As a result of the numerical response of *Orius*, *T. palmi* populations are suppressed in home gardens which are rarely sprayed with insecticides. The occurrence of alternative prey of *Orius* also plays an important role in the naturally-occurring biological control of *T. palmi* because *Orius* can build up their population by consuming other thrips before the occurrence of *T. palmi* on field eggplant. Abiotic factors, such as typhoon and biotic factors, such as spider predation are suggested to reduce field populations of *Orius*. In greenhouses, *Orius* have often been used for the biological control of *Frankliniella occidentalis*, but the population dynamics of *Orius* are not fully understood. Factors influencing the population buildup and persistence of *Orius* in greenhouses are discussed.

20-055

CHARACTERISTICS OF PREDATION BY *ORIOS*Nagai K., E. Yano¹Okayama Prefectural Agricultural Experiment Station, San'yo-cho, Okayama, Japan. ¹Div. Entomol. National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan.

The predatory responses of *Orius* species to prey species and prey stages vary with species or biotypes of *Orius*. In order to use *Orius* in biological control or IPM, it is necessary to know predatory characteristics of *Orius* species against major insect pests on a crop.

Thrips, aphids and spider mites are common and often concurrent pests on many kinds of fruit vegetables. However, sufficient informations about predation by *Orius* are often lacking.

Thrips palmi is the most serious insect pest of eggplant in Japan. Japanese growers apply insecticides frequently to control thrips. *Orius sauteri* is the most dominant and abundant species in vegetable fields in Japan. Therefore, characteristics of predations of this species were studied to facilitate the biological control of *T. palmi* on eggplants.

O. sauteri fed on thrips, aphids, spider mites and other arthropods and attacked thrips most frequently on eggplants in open field. The predatory responses of *O. sauteri* to major insect pests, *T. palmi*, *Aphis gossypii* and *Tetranychus kanzawai*, were investigated under laboratory conditions. The results suggested that *O. sauteri* is an effective predator of *T. palmi* on eggplants in open fields.

In order to develop a simulation model for evaluating the biological control of *T. palmi* by *O. sauteri* in greenhouses, predatory parameters by this species were estimated. The total number fed in the nymphal stage of *O. sauteri* was 203 second instar larvae of *T. palmi* at 25 °C, and the functional responses of all stages of *O. sauteri* to larvae and adults of *T. palmi* were close to Holling's type II curve.

If invited speaker, indicate title of session and name(s) of organizer(s):
20S-5 *Orius* spp. and Other Hemipterans for Biological Control of Agricultural Pests. E. Yano, Y. Hirose (Japan)

20-056

POPULATION INTERACTION BETWEEN ORIUS AND THRIPS IN GREENHOUSES.

E.Yano, K. Nagai¹, P.J.M. Mols², H.J.W. van Roermund², J.C. van Lenteren²

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Orius spp. are mentioned as important predators of thrips, aphids and eggs of lepidopteran insects in the field. In Europe, *Orius* spp. are released for controlling *Frankliniella occidentalis* and *Thrips tabaci* in greenhouses. *Orius* spp. have shown to suppress *F. occidentalis* very quickly in greenhouses because both *Orius* spp. and the thrips concentrate on flowers. By contrast, *Thrips palmi*, the most serious thrips pest in Japan, is mainly found on leaves in greenhouse eggplants. An indigenous *Orius*, *O. sauteri*, is known as an effective predator against *T. palmi* on eggplants in the field. Therefore, use of this *Orius* on greenhouse eggplants is expected in Japan. A simulation model was developed to evaluate the biological control of *T. palmi* with *O. sauteri* on greenhouse eggplants based on a general simulation program "INSIM". The simulation results suggest the importance of early release and high initial density ratio of *O. sauteri* to *T. palmi*. Effects of cropping season and host plant on the performance of *O. sauteri* were also evaluated. *O. sauteri* seems to be effective in warm seasons. The biological control with this species is thought to be more difficult on cucumbers than on eggplants or sweet peppers.

20-058

USE OF TWO POLYPHAGOUS MIRID PREDATORS IN INOCULATIVE BIOLOGICAL CONTROL OF VEGETABLE PESTS

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Macrolophus caliginosus Wagner and *Dicyphus tamaninii* Wagner (Heteroptera: Miridae) are two native generalist predators being successfully used for Greenhouse Whitefly (*Trialeurodes vaporariorum* Westwood) management in outdoor tomato crops in Catalonia, Spain. Both predators also colonize greenhouses and contribute to biological control. In this paper we examine the effectiveness of inoculative releases of *D. tamaninii* and *M. caliginosus* in controlling Greenhouse Whitefly and Western Flower Thrips (*Frankliniella occidentalis* Pergande) on tomato or cucumber field-cages and greenhouse trials with varying predator: prey ratios. The potential of these polyphagous predators for inoculative or augmentative biological control is emphasized.

20-057

Biological control of western flower thrips, *Frankliniella occidentalis* with *Orius* spp. in greenhouses

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Over the last ten to fifteen years western flower thrips (WFT) has developed as a severe pest on both greenhouse ornamentals and vegetables. Natural enemies affecting greenhouse populations of WFT include predatory mites (*Amblyseius* spp.) and bugs (*Orius* spp.), parasitoids (*Ceranisus* spp.) and insectpathogenic fungi (*Entomophthora* spp., *Verticillium lecanii*). *Orius* spp. prey on WFT belonging to the first, second and adult stage and may also feed on other insects as well as pollen. Besides, adults disperse well in the greenhouse. Several *Orius* spp. have been introduced in greenhouses, among others *O. majusculus*, *O. niger*, *O. laevigatus*, *O. albidipennis*, *O. tristicolor* and *O. insidiosus*. Some of these species were difficult to mass rear. Good results have been reported with *O. insidiosus* and *O. laevigatus* on sweet pepper, gerbera and chrysanthemum. In cucumber with pollen being absent, however, *O. majusculus* seems to do better, although this species from Northern Europe has shown to be more diapause sensitive than, for instance, *O. laevigatus* from Southern Europe. *Orius* spp. may show an ovipositional preference for certain positions on host plants. When given a choice *O. insidiosus* will avoid laying eggs on roses which indicates that besides pollen production also other host plant characteristics influence the effect of *Orius* releases. Due to its polyphagous nature also interactions with other natural enemies and pests may occur.

20-059

PHYLOGENY OF APHELINIDAE

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A dataset consisting of 75 morphological characters was scored for some 56 taxa of Aphelinidae and several outgroups. All genera of Aphelinidae were included, including some undescribed genera known to the authors. All genera currently placed in Eriaporinae were included, at least initially, as were various problem taxa such as *Cales*. In cases in which more than one state of a character was known for a genus, the group was treated as two or more terminal taxa.

Characters were taken from all body regions and included both internal and external features. Character systems that are relatively new to aphelinid systematics include features of the head, mouthparts and internal skeleto musculature associated with the prepectus and mesofurca.

Parsimony analysis was performed using heuristic search algorithms in PAUP Version 4.0. Results are somewhat unstable, in part due to effects of various outgroups on character polarity. Uncertainty regarding relationships of Aphelinidae to other Chalcidoidea remains a serious problem. Coccophaginae including Prospaltellinae and Phycsinac is clearly monophyletic, based in part on conformation of the mesofurca and associated musculature. Aphelininae including Aphytinae is monophyletic based in part on presence of linea calva and epicoxal pads under fore coxae. Azotinae is probably part of the aphelinine lineage, as is *Eriaphytis*.

Results indicate that heteronomous host relationships are characteristic of a single lineage of Aphelinidae. Evolution of particular forms of heteronomous biology in Aphelinidae is examined in the context of phylogenetic relationships.

20-060

CYTOTAXONOMIC STUDIES ON SOME SPECIES OF *ENCARSIA* FÖRSTER AND OTHER APHELINIDAE

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The present knowledge on the cytotaxonomy of Aphelinidae is outlined. A chromosome study has been carried out on 13 species of *Encarsia* Förster (*E. asterobemisiae* Viggiani et Mazzone, *E. lutea* -Masi-, *E. lahorensis* -Howard-, *E. leucaspidis* -Mercet-, *E. partenopea* Masi, *E. tricolor* Förster, *E. pergandiella* Howard, *E. aspidioticola* -Mercet-, *E. berlesei* -Howard-, *E. citrina* -Craw-, *E. formosa* Gahan, *E. meritoria* Gahan, *E. transvena* -Timberlake-) and the karyotypes have been reconstructed; centromeric index (C.I.) and relative length (R.L.) has been assessed. Moreover, preliminary studies on chromosome banding carried out for all the species, show that it is possible to distinguish between karyotypes with the same chromosome number and shape; the G-banding of *E. partenopea*, confirms the possibility to use this chromosome attribute to accurately identify pairs of homologues for advanced genetic studies, particularly in taxa lacking polythene chromosomes.

All the available data seem to show that in *Encarsia*, the karyotype evolution consists in a series of robertsonian centric and tandem fusion-fission rearrangements except in *E. lutea* and *E. asterobemisiae*. The data obtained on these two species lead to the conclusion that the chromosome sets of the *lutea* complex might have evolved by different mechanisms such as polyploidy or increasing in the content of heterochromatin, or both.

Once again the importance of karyological data in systematic studies is outlined.

20-061

FUNICULAR GLANDS IN APHELINIDAE AND THEIR SIGNIFICANCE

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The male antenna of a few aphelinids shows, on one or more funicular segments, peculiar cuticular modifications, previously retained "sensillar complexes".

The external features of several types of these structures, found in some species of *Encarsia* Förster, are illustrated.

An ultrastructural study on *Encarsia asterobemisiae* Viggiani et Mazzone and related species demonstrated the association of these presumed "sensillar complexes" with underlying integumentary glands.

According to preceding studies, these secretory elements play a fundamental role in courtship and mating behaviour.

The taxonomic importance of these structures is discussed.

20-062

ESTIMATING ATTACK RATES BY THE PARASITOID APHYTIS

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Aphytis melinus appears to be the major biological control agent of California red scale, a pest of citrus, in Southern California. We are developing a detailed individual-based population model of the interaction that requires independent estimates of per head attack rate by Aphytis in the field throughout the year. An added difficulty is that Aphytis is a host-feeding parasitoid that matures new eggs throughout its life if host meals are available.

This talk will describe a method we have developed and tested for obtaining these estimates. In the fall, when scale was most abundant, it appears that Aphytis was producing eggs and parasitizing hosts at the maximum temperature-dependent rate. This is not expected if it is assumed that Aphytis controlled the scale population at that time.

20-063

SWEET REWARDS: TROPHOBIOSIS AND NATURAL ENEMIES

M. Rose

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The so-called Argentine ant, *Iridomyrmex humilis* (Mayr), utilizes homopteran honeydew as a major food source. The woolly whitefly, *Aleurothrixus floccosus* (Maskell), an international pest of citrus, produces copious amounts of honeydew. On southern California citrus the trophobiotic relationship of these reunited exotic invaders affects the relative abundance of *A. floccosus* parasites. The roles and interactions of *I. humilis* and *A. floccosus*, and the imported parasites *Cales noacki* Howard, *Eretmocerus* Haldeman, and *Amitus spiniferus* Brethes, are discussed.

20-064

CANNIBALISM IN *APHYTIS*S. Ben-Shalom, Y. Rössler¹, D. Rosen

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Wasps of the genus *Aphytis* Howard (Hymenoptera: Aphelinidae) develop as primary, gregarious ectoparasitoids of armored scale insects (Homoptera: Diaspididae). We have studied the ecology of intra-specific competition among the developing larvae of a uniparental strain of *A. lingnanensis* Compere, reared on the oleander scale, *Aspidiotus nerii* Bouché. A clutch-size manipulation experiment was performed by transferring the parasitoid immature stages, at various densities, onto unparasitized hosts. Intra-specific competition increased as clutch size increased. Monitoring larval development on a daily basis indicated that the only density-dependent mortality factor was intra-specific predation (i.e., cannibalism). Lower rates of cannibalism were found in clutches where all individuals were sibs than in clutches composed of non-sibs. This suggested that sib-recognition occurred among the larval stages of *Aphytis*. Adult size was strongly influenced by the intensity of cannibalism; thus, non-sibs were larger than sibs. Female larvae exhibited a high degree of cannibalism toward males, causing a female-biased secondary sex ratio as rates of cannibalism increased.

20-066

DUAL ONTOGENY ON *ENCARSIA PORTERI* (MERCET) (HYMENOPTERA: APHELINIDAE) SPECIALLY RELATED TO PARASITISM OF LEPIDOPTERAN EGGS

E. A. LOBOS, A. E. DARCHUK

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In Argentina, *Encarsia porteri* (Mercet) is found in cotton crops, parasitizing *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae), *Alabama argillacea* Hubner (cotton leafworm-CLW) and *Helicoverpa gelotopoeon* Dyar (cotton bollworm-CBW) (Lepidoptera: Noctuidae).

Laboratory studies were carried out (25 °C, HR 65-70%) to find out the biology of males of *E. porteri* and the preference of the non-copulated females for lepidopteran host.

In CLW eggs (n=42), the duration of the pre-adult instars of *E. porteri* was: eggs: 2.5; larvae I-III: 3.9 and male pupae: 7.7 days respectively. It took 14.33 (SD=1.04) days from parasitization to the emergence of adult. *E. porteri* females (n=10) parasitized an average of 19 (SD=14.86) eggs of *A. argillacea*. Aphelinids females (n=10) significantly prefer to parasitize eggs of CLW rather than of CBW (t_{0.5,9}). Percentages of parasitism were 28.12 and 3.75 % respectively. The rate parasitized eggs/contacted eggs was 0.49 for the CLW and 0.16 for the CBW.

Field studies (28° 5' S; 64° 10' O), were carried out during 1992/3 and 1993/94 in order to understand the seasonal parasitism of the oophagous found in cotton crops.

E. porteri is found from December though February. The proportion of parasitized eggs of *A. argillacea* the values of the proportions were 3% (n=1844) and 14 % (n=1319) respectively. For *H. gelotopoeon* (n=189 and n=425 respectively) the values of the proportions were 1% lower in both periods. All aphelinids emerged from parasitized eggs were male.

Among the eggs of CLW collected in 1993/94, there was one from which both a male of *E. porteri* and a female of *Trichogramma pretiosum* Riley. From another eight black eggs, which had signs of having been parasitized by *T. pretiosum*, aphelinids males emerged. Apparently, the high parasitism rate and the decrease in the density of host eggs determined conditions for both parasitoids to compete.

20-065

OVIPOSITION MECHANISMS IN THE WHITEFLY PARASITOID, *ENCARSIA* AND *ERETMOCERUS* (HYMENOPTERA: APHELINIDAE)

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Species of the aphelinid genera *Encarsia* and *Eretmocerus* both parasitize whiteflies but their development is quite different, the former being endoparasitoids which develop from eggs laid within the host whereas the latter develops as 'pseudo' endoparasitoids from an egg laid external to (underneath) the host. We investigated the morphology of the ovipositors of these two taxa using scanning and transmission electron microscopy.

The apex of the dorsal ovipositor valve (gonapophyses of 9th abdominal segment) of the endoparasitic *Encarsia transvena* was found to be sharply pointed and well-sclerotized and therefore adapted for penetration through the host cuticle. In contrast, that of *Eretmocerus mundus* was atypical in that it formed a flat and apparently flexible pad that is likely to make penetration of the host cuticle unlikely. These and other features of the ovipositors are illustrated and described in detail.

20-067

PARTHENOGENESIS-INDUCING MICROORGANISMS IN *APHYTIS* AND OTHER APHELINIDSE. Zchori-Fein, Y. Gottlieb, R. T. Roush¹, J. H. Werren², D. Rosen

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In parasitic Hymenoptera, severe sex ratio distortion are sometimes associated with the presence of symbiotic microorganisms. Symbiont-induced uniparental reproduction has been reported from several families in the Chalcidoidea. About one fourth of all species of the aphelinid genus *Aphytis* exhibit thelytokous parthenogenesis. After antibiotic treatments successfully induced the production of male in uniparental lines of *Aphytis*, various aspects of the relationships between the wasps and their symbionts were studied: 1) The systematic status of the parthenogenesis-inducing microorganisms was determined by using two genes (16s rDNA and *ftsZ*) for the construction of a phylogenetic tree. 2) Electron microscopy as well as confocal microscopy were used to confirm the presence of microorganisms in uniparental lines of *Aphytis* and to follow the distribution pattern of the symbionts in the ovaries and eggs. The analyzed data assign the symbionts to the genus *Wolbachia* and show that *Wolbachia* from different *Aphytis* species are almost identical. Results suggest that *Wolbachia* move from nurse cell to developing oocytes. In freshly-laid eggs, the symbionts are concentrated in the posterior pole, and in later stages of embryogenesis they are found surrounding nuclei throughout the embryo.

20-068

POPULATION BIOLOGY AND BEHAVIOR OF *APHELINUS ASYCHIS* WALKER AND *APHELINUS VARIPES* (HYMENOPTERA: APHELINIDAE), MAJOR PARASITOIDS OF *DIURAPHIS NOXIA* (MORDVILKO) (HOMOPTERA: APHIDIDAE)

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We describe the distribution of *Aphelinus asychis* and *Aphelinus varipes* on *Diuraphis noxia* throughout Eurasia. We discuss the spatial and temporal dynamics of *D. noxia* parasitism in southern France. We present results on host defense behavior, parasitoid search, and genetics of host specificity to distinguish among hypotheses concerning the low number of *D. noxia* parasitized per colony. We summarize results on the mating-finding behavior and dispersal of *A. asychis* and present mathematical models of populations dynamics that incorporate these results. We discuss the implications of these models for establishment of introduced populations and describe field experiments to test these implications. We summarize results on the quantitative genetics of *A. asychis* fitness components which show no directional changes during long-term laboratory rearing but suggest that drift does occur. We compare fitness components for crosses within and between cultures derived from collections of *A. asychis* throughout Eurasia. These crosses show differences in fitness components within, but not among, cultures. The crosses revealed reproductive incompatibility among cultures from different geographic regions; which subsequent experiments showed was caused by lack of sperm transfer. We discuss the implications of our research for the design of biological control introductions.

20-070

APHELINID PARASITOIDS AS SUSTAINABLE BIOLOGICAL CONTROL AGENTS IN GREENHOUSES

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Aphelinid parasitoids have been used for control of pests in greenhouses since the 1920s, but large scale application started only some 25 years ago. A success story is the biological control of greenhouse whitefly, *Trialeurodes vaporariorum*, with the aphelinid *Encarsia formosa*. Nowadays this parasitoid is applied on 5000 hectares of vegetable crops in most countries with a reasonable greenhouse industry. Weekly, more than 20 million individuals of *E. formosa* are mass reared and shipped to growers. About 10 years ago, another whitefly species, *Bemisia* sp., developed to pest status. Although *E. formosa* does attack *Bemisia*, it is not capable to reduce *Bemisia* numbers to the same very low levels as it does with greenhouse whitefly. Therefore, a search for more effective natural enemies for *Bemisia* was started. Several other aphelinid species are studied for control of scales and aphids. The following recent developments will be discussed concerning the role of aphelinids in greenhouse biological control:

1. status of commercial biological control with aphelinid parasitoids,
2. survey of aphelinid parasitoids under study,
3. use of preintroductory evaluation methods to select potentially promising aphelinid parasitoids,
4. quality control methods of mass reared aphelinid parasitoids,
5. the issue whether (partial) hyperparasitoids should be imported and released, and
6. the question if parasitoid biotypes studies are useful to identify suitable candidates for biological

20-069

POPULATION DYNAMICS OF THE ARROWHEAD SCALE, *UNASPIS YANONENSIS* (HOMOPTERA: DIASPIDIDAE) UNDER BIOLOGICAL CONTROL

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The arrowhead scale, *Unaspis yanonensis* was one of the serious citrus pests in Japan before the introduction of the two parasitoids, *Aphytis yanonensis* and *Coccobius fulvus* from China. Population dynamics of *U. yanonensis* were studied from 1982 to 1995 at a citrus grove in Fukuoka, Japan. We released only *A. yanonensis* in June, 1982 at the grove and *C. fulvus* invaded from outside of the grove in 1989. The adult female density of *U. yanonensis* decreased rapidly after the release of *A. yanonensis*, which kept the density low level after that. The population density of *U. yanonensis* decreased to further low level after the invasion of *C. fulvus*. Seasonal changes of the host density and the percentage parasitism of the two parasitoids indicate that the two parasitoid perform as complementary natural enemies and both parasitoids contribute to maintain the pest population at lower level than single species introduction.

20-071

STEMBORER ECOLOGY

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The ecology of lepidopterous stemborers of cereal crops (wheat, rice, maize, sorghum, pearl millet, etc.) and sugarcane is briefly reviewed, with particular reference to the most important pest genera of Pyralidae (*Ostrinia* Hübner, *Chilo* Zincken, *Scirpophaga* Treitsche, *Diatraea* Guelding, *Eldana* Walker, *Coniesta* Hampson) and of Noctuidae (*Busseola* Thunberg, *Sesamia* Guenée). The stem-boring habit in Lepidoptera feeding on Poaceae [= Gramineae], Cyperaceae and Typhaceae has evolved polyphyletically over about 25 million years, since the late Tertiary. Pest interactions with cultivated crops and their precursors over the past 10,000 years are therefore comparatively recent. Current interactions of indigenous and non-indigenous stemborer genera with indigenous and non-indigenous host plants indicate dynamic elements that persist, especially through changes in geographical distribution and host range.

Good ecological information on stemborer species and complexes is necessary for effective planning of pest management strategies and is now available for most major species on their main crop hosts. There is however a continuing need for ecological research on stemborers on crops and on non-crop hosts (mostly grasses and sedges), including higher trophic interactions with natural enemies.

20-072

IMPACT OF ECOLOGY AND HOST PLANT SHIFTS OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE) ON ITS BIOLOGICAL CONTROL IN GRAMINACEOUS CROPS

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Biological control of *Eldana saccharina*, although considered one of the better control options in sugarcane and other graminaceous crops in Africa, has been difficult to implement. In addition to the very cryptic nature of *E. saccharina* in these crops, which protects it from a number of more conventional control measures such as pesticide applications, there are a number of other factors complicating establishment of biological control agents.

- It is indigenous to Africa, where it occurs in numerous wetland sedges and grasses. Classical biological control strategies therefore cannot be implemented.
- Even though it was described from sugarcane in Sierra Leone over 100 years ago, it is regarded as a recent invader of sugarcane in especially southern Africa. Here it first reached pest status for a period in 1939, and since 1970 has been of major concern to the South African sugar industry. It is thus unlikely that indigenous natural enemies have had time to colonise this "new" habitat.
- Previous workers have found very few natural enemies, especially parasitoids, in graminaceous crops. The impression has thus been created that *E. saccharina* has very few parasitoids. However, if successful natural enemies were present in the crops investigated, this borer would not be a pest. In addition, no work had been done to investigate the ecology and natural enemy complex of *E. saccharina* in its indigenous host plants. Studies of this nature could thus provide some pointers to its control in crop plants.

This paper discusses pest-host plant association, host plant shift, indigenous pest control and biological control concepts in the light of current knowledge.

20-074

FORAGING STRATEGIES OF GUILDS OF PARASITES AND APPLICATIONS FOR BIOLOGICAL CONTROL

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ABSTRACT NOT RECEIVED

20-073

NOVEL ASSOCIATIONS AND THE NEED FOR ECOLOGICAL AND PHYSIOLOGICAL EQUIVALENCIES
R.N. Wiedenmann (Champaign-United States)

ABSTRACT NOT RECEIVED

20-075

SYSTEMATICS IN STEM BORER BIOCONTROL

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Past and current biocontrol programmes developed against cereal stem boring Lepidoptera have depended for their success on having a sound basis in natural enemy systematics. A great deal of confusion has been apparent in the past, and in many cases has been perpetuated for decades, compounding the effects of misidentifications or misunderstandings. Poor taxonomy can vastly increase the amount of work carried out by biocontrol and IPM practitioners, for example by the reintroduction of agents into areas where they are already present, though known by a different name, or as yet unidentified. Conversely, sound systematics is essential for biocontrol success, and often draws attention to hitherto untapped resources of new potential control agents. The past and current situations regarding stem borer natural enemies are reviewed with specific examples, and suggestions for future research, and in particular future collaboration between systematists and IPM/biocontrol practitioners are proposed.

20-076

BIOLOGICAL CONTROL OF STEM BORERS BY TACHINID FLIES, PAST AND FUTURE

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In the 80 years since the first attempts were made to introduce *Lixophaga diatraeae* Townsend from Cuba to Louisiana for the control of *Diatraea saccharalis* (Fabricius) tachinids have been introduced into fifty countries for the control of gramineous stem borers with varying degrees of success. In addition to classical biological control other strategies involving inoculative and inundative releases, mass production of tachinids on natural and factitious hosts, genetic improvement and amelioration of the microhabitat have been attempted. These as well as the status of current major programs are reviewed to determine the direction of future research and utilization of tachinids as control agents of stem borers.

20-078

USE OF *TRICHOGRAMMA*, ESPECIALLY AGAINST *OSTRINIA*

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Trichogramma are the most widely augmented species of natural enemy worldwide. Strategies for implementation of *Trichogramma* use are also the most diverse of any augmented natural enemy. Production facilities have been adapted to fit into a variety of socioeconomic conditions. These range from outdoor labor-intensive insectaries that provide *Trichogramma* to local surrounding farms, to mechanized biofactories producing and marketing commercial products for input-intensive industrial farming. *Trichogramma* releases have also been adapted to fit into a wide variety of agricultural practices. Perhaps the best example of this is the implementation of *Trichogramma* for *Ostrinia* suppression in western Europe and North America. Implementation research efforts on *Ostrinia* suppression with *Trichogramma* in these regions have focussed recently on quality assurance, formulation, and delivery systems. These efforts have resulted in *Trichogramma* products which possess attributes similar to those of insecticides, such as long "shelf-life", long residual activity, and ability to be applied with conventional equipment.

20-077

PARASITIDS OF CONCEALED EGGS OF NOCTUID STEMBORERS AND THEIR POTENTIAL IN BIOLOGICAL CONTROL (HYMENOPTERA: SCELIONIDAE, TRICHOGRAMMATIDAE; LEPIDOPTERA: NOCTUIDAE)

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Several species of noctuid stemborers, mostly belonging to *Sesamia*, lay their egg masses between the leaf sheath and the stem of cultivated and wild Graminae. The egg masses in such concealed, or somewhat exposed, oviposition sites are directly attacked by trichogrammatids, which have a negligible impact, and by scelionids belonging to the genus *Telenomus*, which may exert an effective natural control.

Telenomus species are of two different groups according to their morphological adaptation and attack strategy. The "crawlers" (e.g. *T. busseolae*), which have a flattened body, reach the eggs by crawling between leaf blade and stem, and the "drillers" (e.g. *T. cirphivorus*), which are not flattened, lay their eggs by drilling with the ovipositor through the leaf sheath. Their host associations and effectiveness are reviewed and discussed. Rearing techniques of *S. nonagrioides* and *T. busseolae* and preliminary results on host recognition cues are described. Taxonomic revision for undescribed reared "drillers" and for described species from unknown hosts and possibly belonging to "crawlers", is suggested.

20-079

BIOLOGICAL CONTROL OF THE NEOTROPICAL DIATRAEA AND REOATED GENERA PF STEMBORERS
L.A. Rodrigues-del-Bosque (Taumalipas-Mexico)

ABSTRACT NOT RECEIVED

20-080

BIOLOGICAL CONTROL EFFORTS AGAINST THE CEREAL STEM BORERS, *BUSSEOLA FUSCA* AND *CHILO PARTELLUS* IN SOUTH AFRICA

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The most important pests of cereal crops in South Africa are the stem borers, *Busseola fusca* (Fuller) (Lep. : Noctuidae) which is indigenous, and *Chilo partellus* (Swinhoe) (Lep. : Pyralidae) which invaded Africa from India.

In South Africa 18 indigenous parasitoids were recorded from *B. fusca* and 13 parasitoids from *C. partellus*. These parasitoids reduce pest populations but are not able to prevent economic damage.

During the last 20 years several attempts have been made to augment the fauna of the local parasitoids of stem borers by introductions of exotic parasitoids. The following parasitoids were introduced: *Allorhogas pyralophagus* (Marsh) (Braconidae), *Cotesia flavipes* (Cameron) and *C. chilonis* (Munakata) (Braconidae), *Descampsina sesamiae* Mensil (Tachinidae), *Mallochia pyralidis* Wharton (Ichneumonidae), *Meloboris sinicus* (Holmgren) (Ichneumonidae), *Metagonistylum minense* Townsend (Tachinidae), *Paratheresia claripalpis* Van der Wulp (Tachinidae), *Sturmiopsis inferens* Townsend (Tachinidae), *Tetrastichus howardi* (Olliff) (Eulophidae), *Trichogramma chilonis* Ishii and *T. ostrinia* Pang & Chen (Trichogrammatidae), and *Xanthopimpla stemmator* Thunberg (Ichneumonidae).

Several recoveries of introduced parasitoids have been made from field collected stem borers but the impact on the local pest populations was negligible.

20-082

ENVIRONMENTAL ISSUES CONCERNING THE IMPORTATION OF NON-INDIGENOUS BIOCONTROL AGENTSF.G. Howarth

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The importation and release of non-indigenous organisms to control pest populations continues to be promoted as environmentally safe; however, accumulating evidence from past and current projects raises serious concerns. Introduced biocontrol agents have been implicated in the extinction of numerous nontarget species, disrupted native ecosystems, and affected human welfare. Of greatest concern is species extinctions, especially on islands (including numerous species of land snails, insects, birds, and reptiles), but severe negative impacts also occur in continental areas. Many effects were discovered serendipitously by persons conducting fieldwork at the right time and place, thus many impacts escaped notice and remain undocumented.

The technology is as highly unnatural and anthropocentric as is the use of chemical pesticides and should be recognized as artificial rather than natural biocontrol. Persons releasing organisms beyond their natural range undertake a grave responsibility; once released these agents are pervasive (i.e., able to disperse far beyond the target system), permanent (i.e., able to multiply and evolve in perpetuity), injurious (i.e., able to harm nontarget organisms), and insidious (i.e., able to affect ecosystems, sometimes in profound ways). Long-term ecological studies are needed on both the efficacy and environmental impacts of biocontrol introductions to make applied ecology more predictive and to produce more appropriate protocols governing proposed introductions. Given the risks, more open and comprehensive public oversight and regulation of all proposals to introduce and release non-indigenous organisms is required.

20-081

RELEASES OF EXOTIC PARASITIDS AGAINST EXOTIC AND NATIVE STEMBORERS IN AFRICA AND NEIGHBORING INDIAN OCEAN ISLANDSW. A. Overholt

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There have been many attempts over the past fifty years to suppress populations of exotic and native lepidopteran stem borers in Africa and neighboring Indian Ocean Islands through the introduction of exotic parasitoids. Several parasitoids have been successfully established in the Mascarene Islands and in Madagascar, but there were no successes reported from mainland Africa until very recently when the exotic parasitoid, *Cotesia flavipes* Cameron, was introduced into Kenya. All the parasitoids that have established on the Indian Ocean Islands and in Kenya are thought to represent old parasitoid/host associations, and *Cotesia* spp. represent a disproportionate share of the establishments. Possible factors which have influenced the establishment of exotic parasitoids and implications for future biological control programmes are discussed.

20-083

ENTOMOLOGIST AS PIVOTAL HONEST BROKER IN THE BIOCONTROL-INSECT CONSERVATION DEBATEM.J. Samways

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Population decline, range shrinkage, range lacunae and species extinction have characterized the increasing intensity of landscape fragmentation. Biocontrol practitioners have both exacerbated and slowed this attrition of biodiversity. Conservationists, in contrast, unequivocally aim to maintain biodiversity, yet have made few positive suggestions which rationalize species preservation and biotic pest control. A polarized and, at times, unscientific, debate has developed.

Have we really weighed the relative risks and advantages of biocontrol, from the view that it is inevitably going to be more widely practised? It seems as though a graded risk strategy is our only option. If so, what form should it take? A way forward is suggested, with special reference to South African examples.

20-084

HARVESTING SAFELY FROM BIODIVERSITY: NATURAL ENEMIES AS SUSTAINABLE AND ENVIRONMENTALLY FRIENDLY SOLUTIONS FOR PEST CONTROL

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A study of the European biological control literature leads to the conclusion that importation of arthropods for biological control of pest arthropods has not led to unwanted side-effects. Current pest control policies in many European countries support or stimulate biological control, and natural enemy imports increased during the past three decades. At the same time development of rules takes place, making import and release of non-indigenous organisms very difficult. It is of great priority to find a balance between reasonable regulation of importation of new candidates for biological control and the possibility to develop sustainable, environmentally safe pest control.

Use of non-indigenous micro-organisms for biocontrol is covered by the national registration procedure of each European country. For macro-organisms, European countries have very different criteria to allow importation and release. They range from no criteria at all to rather strict criteria including information on potential environmental impacts. Harmonization for importation of non-indigenous organisms is under discussion within the EU, and there are efforts to include elements of the FAO Code of Conduct for the Importation and Release of Biological Control Agents into the EU regulation. Regulation of importation of natural enemies may be needed, but such ruling should preferably be developed by scientist and biocontrol practitioners, not by administrators. Very strict regulation procedures for biocontrol agents will result in more damage for the environment because of the continued use of chemical pest control and demotivates those with an honest approach for developing environmentally friendlier alternatives.

20-086

RESPONSE OF 3 HYMENOPTERAN PARASITOIDS INTRODUCED FOR FRUIT FLY CONTROL TO A NON-TARGET TEPHRITID, *PROCECIDOCHARES ALANAE* (DIPTERA: TEPHRITIDAE): IMPLICATIONS FOR BIOLOGICAL CONTROL IN HAWAII

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The response of 3 larval-pupal parasitoids, *Diachasmimorpha longicaudata* (Ashmead), *D. tryoni* (Cameron), and *Tetrastichus giffardianus* Silvestri to the pamakani gall fly, *Procecidochares alanae* L. was determined in the laboratory. We also observed responses of these parasitoids to their normal rearing hosts, *Bactrocera dorsalis* (Hendel), and *Ceratitidis capitata* (Weidemann). *D. tryoni* landed on pamakani galls or on dishes containing *P. alanae* larvae as frequently as on dishes containing *C. capitata*. In contrast, *D. longicaudata* only rarely landed on the galls. *D. tryoni* and *D. longicaudata* rarely oviposited in galls (< 1% of observations). *D. longicaudata* probed in *P. alanae* larvae in the dishes as frequently as in *B. dorsalis* larvae, but no parasitoid offspring were observed. Ovipositional probes of *D. tryoni* were more frequently observed in *C. capitata* than all other substrates. No *D. tryoni* developed in gall fly larvae. *T. giffardianus* landed on pamakani galls and *P. alanae* larvae as often as on *B. dorsalis*. However, no *T. giffardianus* were observed entering galls with artificially opened windows. A 1 year field survey was done to determine the abundance of parasitoids of *P. alanae*. None of the parasitoids recovered were species introduced for control of the pest tephritids in Hawaii. The following 4 species were recovered: *Eupehmus cushmani* (Crawford), *Eurytoma tephritidis* Fullaway, an unidentified torymid and pteromalid. We discuss the implications of our results for future augmentative or classical biological control studies.

20-085

IMPLICATIONS OF NON-TARGET EFFECTS OF INTRODUCED BIOLOGICAL CONTROL AGENTS PARTICULARLY WITH RESPECT TO BENEFICIAL SPECIES

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A New Zealand research programme has been initiated to evaluate the effects of entomophagous biological control introductions on non-target species, and to improve quarantine protocols for regulatory authorities to implement prior to approving new introductions. As a model, methods are being developed to assess and monitor the impact on non-target organisms of *Microctonus aethiopoides* Loan and *M. hyperodae* Loan (Hymenoptera: Braconidae), parasitoids already released to control the adult stage of the introduced forage pests *Sitona discoideus* Gyllenhal and *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) respectively. Field surveys have shown that many species of taxonomically related native weevils occur in mixed populations with the target hosts. Laboratory host range tests have been carried out on some of these species showing that *M. aethiopoides* achieved higher levels of parasitism in native species than *M. hyperodae*, indicating that it may be a more polyphagous parasitoid species. Monitoring field populations of non-target weevil species has so far supported this finding, with 13 non-target species having been found parasitised by *M. aethiopoides*, and one by *M. hyperodae*.

Studies have shown that one of the unintended hosts of *M. aethiopoides* is *Rhinocyllus conicus* (Froehlich), a weed biological control agent introduced to control nodding thistle. Current research is evaluating the effect of parasitism on *R. conicus* survival and fecundity. While *R. conicus* was exposed to *M. aethiopoides* in limited host range tests before release in 1982, no parasitism was detected. This raises the question of the design of adequate laboratory tests, and also the potential for parasitoids to become 'conditioned' to alternative non-target hosts.

20-087

ETHICAL STANDARDS AND CONCERNS OF INTERNATIONAL BIOLOGICAL CONTROL

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A system of environmental ethics recently developed by Lawrence Johnson is integrated with standards of professional ethics to analyze the moral implications of biological control technologies. Entities are morally relevant when they possess wellbeing interests (i.e., functions or processes that can be better or worse in so far as the entity is concerned) and species/ecosystems are morally relevant because they are not simply aggregates of individuals, so their processes, properties and wellbeing interests are not reducible to the sum of their individual members. Following Johnson's thesis, species and ecosystems have morally relevant interests in surviving and maintaining themselves as integrated wholes with particular self-identities. This theoretical structure gives rise to a number of ethical criteria that are particularly relevant to practitioners of biological control, who are professionally obligated to recognize these constraints. These criteria apply to the ecosystem (the extent to which it is large, native, unique, and integrated) and to the action being considered (the extent to which it is novel, omnipresent, monitored, reversible, and necessary). In these terms, it is evident that not all biological control efforts are ethically defensible. In general terms, natural biological control is most desirable, followed by augmentative strategies, classical approaches, and finally neoclassical biological control. Specific cases are examined to illustrate the ethical concerns. Finally, it is argued that formalized restrictions of biological control are necessary, given the ethical obligations of the scientific community and the unique properties of biological control technologies.

20-088

ENSURING THE QUALITY, SAFETY AND FAIRNESS OF
INTERNATIONAL BIOLOGICAL CONTROL

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Alien invasive species are a growing problem worldwide: increased trade has accelerated the appearance of alien pests, traditional threats to agriculture are intensified by the potential to exploit alien pests as non-tariff trade barriers, and the importance of alien species as risks to biodiversity conservation is generating growing concern in the environmental community. Classical biological control (CBC) has the potential to contribute to the management of these problems. However, as concerned stakeholders in CBC expand from a traditional, small scientific community in a few, developed countries to include new countries, non-scientists and the public at large, there is a need for greater awareness and understanding of CBC and for consensus on procedures for its safe and fair implementation. Some recent international agreements suggest how this can begin, but there is more to be done.

20-089

NON-INDIGENOUS SPECIES AND BIOLOGICAL
CONTROL AND BIOLOGICAL AND REGULATORY
ISSUES, A CALIFORNIA PERSPECTIVE

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California is a microcosm of most of the issues and concerns involved in the introduction of non-indigenous organism, their regulation and their management. Unlike the old world, the period over which California's landscape and economic interests have been affected by non-indigenous organisms has been brief. It began with the establishment of the Jesuit Missions along California's coast in the late 1700s and remains largely unabated today because of the global economy and international trade.

Initially, the regulatory concerns about non-indigenous organisms arose because of agricultural interests. Other regulations have followed, both State and Federal, and they have sought to prevent the introduction of a variety of organisms, largely based on special economic interests. More recently, environmental concerns have entered the regulatory arena. Generally, the strategy of regulation has focused on developing specific lists of taxa that cannot be imported or released, rather than on a process of evaluating the risk or benefit of an introduction. This has lead to a hodgepodge of poorly coordinated regulations that have evolved by accretion. The current approach has been of limited effectiveness. Its reappraisal is sorely needed. These issues affect biological control's practices and procedures. We have a professional and ethical obligation not to exacerbate the problem of non-indigenous organisms.

20-090

INBREEDING EFFECTS ON THE DEVELOPMENT AND REPRODUCTION OF THE PREDATOR *PODISUS MACULIVENTRIS* (SAY) (HETEROPTERA: PENTATOMIDAE)

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The predatory pentatomid *Podisus maculiventris* (Say) has been recognized for its potential as a biocontrol agent of various lepidopterous and coleopterous crop pests. The current study focusses on the effects of inbreeding in the laboratory culturing of this beneficial insect.

Several developmental and reproductive parameters were measured throughout 15 generations of laboratory rearing to compare the quality of a reference population with that of two inbred populations. The latter were started from a small number of sister-brother founders and were subsequently kept at low population size. Predators in all colonies received caterpillars of the greater wax moth, *Galleria mellonella* L., and of the beet armyworm, *Spodoptera exigua* (Hübner), as food. Developmental and reproductive traits did not vary dramatically between the reference and inbreeding treatments. Nonetheless, weight and hatching of eggs, developmental rate and survival of nymphs, and total fecundity of females were consistently higher in the reference population than in both inbred populations. Weight and size of adults in the reference population, however, were intermediate between those of adults in the two inbred populations. No significant relationship between weight or size of female adults and their total fecundity could be established in laboratory-reared *P. maculiventris*.

20-092

ACCEPTANCE AND SUITABILITY OF UV-IRRADIATED *HELICOVERPA ARMIGERA* (HÜBNER) EGGS FOR *TRICHOGRAMMA CHILONIS* ISHII

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Helicoverpa armigera (Hübner) eggs were irradiated with UV-light. Egg mortality increased with duration of exposure to the UV-light source; 98% of eggs were killed with approximately 50 min exposure. Eggs were treated for 60 or 90 minutes and offered to *Trichogramma chilonis* Ishii females together with control eggs. Irradiated eggs were as readily accepted as untreated eggs. While clutch size and sex ratio of the progeny were not affected by the treatment, mortality of the developing parasitoid was higher in UV-irradiated eggs.

The different parasitization phases of *T. chilonis* females were observed on the first egg accepted on irradiated and untreated eggs in a no choice test. Drumming, oviposition and total time spent per host showed no difference but drilling time was significantly shorter on irradiated eggs. It seems, that morphological changes in the egg chorion have occurred during the treatment. These changes could be responsible for the higher parasitoid mortality in irradiated eggs, as the chorion regulates the water and gas exchange between egg and environment.

20-091

REARING THE GYPSY MOTH PREDATOR, *CALOSOMA SYCOPHANTA* L. ON AN ARTIFICIAL DIET (LEPIDOPTERA: LYMANTRIIDAE - COLEOPTERA: CARABIDAE),

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Calosoma sycophanta L. is an important specialist predator of the gypsy moth in North America. It has been released in areas of the United States that gypsy moths have recently invaded, and there is interest in making further releases. Until recently, it has been necessary to rear the beetle on gypsy moths supplied by personnel at the US Department of Agriculture APHIS Methods Development Laboratory in Massachusetts, USA. An artificial diet would make rearing the beetles more economical. A diet for larval beetles has been developed, consisting of ground beef liver and chicken. Larvae can be reared from egg to adult on this diet, and the characteristics of the resulting adults compare favorably to beetles reared on gypsy moth pupae. An adult diet is also being developed because adult beetles must feed heavily on gypsy moth larvae in order to reproduce.

20-093

IN VITRO CULTURE OF THE PUPAL PARASITOID *BRACHYMERIA INTERMEDIA* (NEES) (HYMENOPTERA: CHALCIDIDAE)

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Brachymeria intermedia, a polyphagous solitary endoparasitoid of lepidopterous pupae, was reared from the egg to adult stage on artificial media based on commercial veal homogenate for babies (Gerber®) integrated with chicken egg yolk, yeast extract, wheat germ and 10% extract of *Galleria mellonella* L. pupae. The mean adult yield was of about 61%.

Complete parasitoid development was obtained also on diets devoid of host material, i.e. containing only veal homogenate, egg yolk, yeast extract and wheat germ. Adult yields were, however, quite low (22.5%).

The adults appeared normal and were fecund.

20-094

ADVANCES ON *IN VITRO* REARING *TRICHOGRAMMA* SPECIES IN BRAZIL

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Research related to *in vitro* rearing of egg parasitoids in Brazil was initiated in 1990 with the first report in Latin America of the rearing of *Trichogramma pretiosum* on an artificial diet. Although the complete development on a diet composed of hemolymph of *Helicoverpa zea* was obtained, the survivorship and percentage of normal adults were very poor. Mold and/or bacteria contamination were also a limitant factor. In 1993, the addition of bovine fetal serum to this diet allowed the full development of *T. galloi* for the first time. Successive studies with *T. pretiosum* and *T. galloi* showed that both parasitoids differ in their nutritional requirements. The basic medium was composed of larval hemolymph of *H. zea*, egg-yolk, bovine fetal serum, and streptomycine. However, inclusion of egg-juice from *Heliothis virescens* increased the survivorship and adult normalcy of *T. pretiosum*, but not of *T. galloi*. Larval hemolymph of *H. zea* was preferred instead of *Bombyx mori* and pupal holotissues of *Diatraea saccharalis*, *Anticarsia gemmatilis* and *B. mori* because of the best results with the first one. Use of larval hemolymph of *H. zea* resulted in 50.89% and 26.10% of adult emergence for *T. pretiosum* and *T. galloi*, respectively, with a high percentage of normal adults. Wing deformities and an oversized abdome were the most common abnormalities. No deformities were observed on the genital apparatus. Both *in vitro*-reared parasitoids had a reduced parasitism capacity and longevity compared to the *in vivo*-reared ones. Studies on the parasitism behavior, chorionic ultrastructure of the hosts and ovipositional stimulants have been conducted to support the development of an artificial egg using plastic membranes.

20-096

STIMULANTS FOR IMPROVING *IN VITRO* REARING OF *TRICHOGRAMMA* SPP. (HYMENOPTERA: TRICHOGRAMMATIDAE) WITH ARTIFICIAL MEDIA WITHOUT INSECT HEMOLYMPHW.H. Liu¹, S.C. Han¹, Q.X. Chen¹, S. Grenier²¹ Guangdong Entomological Institute, Guangzhou, China² Laboratoire de Biologie Appliqu e, LA INRA, INSALyon, Villeurbanne, France.

Great progress has been made recently in research on *in vitro* rearing of *Trichogramma* spp. in China, France, etc. But, the insect hemolymph, as a main component of the artificial medium, is difficult to obtain in the countries other than China. It is very urgent to solve the problem of artificial diets without insect hemolymph. To improve the oviposition is the most important problem during *in vitro* rearing of *Trichogramma* spp. without insect hemolymph. In this paper, several kinds of chemicals were selected as ovipositional stimulants, among them the polyvinyl alcohol is the most effective and economical one which can be used for increasing the efficiency in oviposition of *Trichogramma brassicae* and *Trichogramma dendrolimi* in artificial eggs. The parasitism was 100%, the average number of trichogrammatid eggs in a capsule was 259.6 ± 97.5 (max. 456, min. 77) and 351.0 ± 98.6 (max. 566, min. 233) respectively. In the same conditions, control (non stimulant) was 27.5 ± 34.0 and 181.9 ± 43.2 , respectively.

20-095

DEVELOPMENT OF A UTILITARIAN, ARTIFICIAL CULTURE MEDIUM AND PRESENTATION SYSTEM SUITABLE FOR REARING MULTIPLE INSECT PREDATORS AND PARASITOIDS

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The poster describes an inexpensive artificial culture medium free of any insect components as well as the production of a simple encapsulation/presentation system. The medium has supported the growth and development of several species of insect predators and parasitoids. Predators that have been successfully reared from egg-adult include several hemipterans (e.g., *Geocoris punctipes*, *Podisus maculiventris*, *Perillus bioculatus*, *Lyctocoris campestris*, and *Xylocoris flavipes*), and coleopterans (e.g., *Calosoma sycophanta* and *Coleomegilla maculata*). Two hymenopterous ectoparasitoids of noctuid pupae also have been successfully reared on the medium (*Diapetimorpha introita* and *Cryptus albitarsus*).

Encapsulation systems for the medium are described, along with optimization of procedures for terminal sterilization of the encapsulated units.

Results on growth, development, fecundity, and host/prey searching behavior of predators and parasitoids reared on the medium are described in accompanying presentations by other authors in the same session.

20-097

NUTRITIONAL STUDIES ON LARVAL DEVELOPMENT OF AN APHIDOPHAGOUS COCCINELLID, *HARMONIA AXYRIDIS* PALLAS — CHEMICALLY DEFINED DIET AND ACTIVE INGREDIENT IN DRONE HONEYBEE POWDER. —K. Nijima, T. Iwasa¹ and M. MatsukaLaboratory of Entomology, Faculty of Agriculture, Tamagawa University, Machida, Tokyo, Japan. ¹ Advanced Technology Laboratory, Kubota Corporation Ryugasaki, Ibaraki, Japan

Chemically defined diets (CDs), which is good to rear aphidophagous lacewings, consisting of amino acids, minerals, vitamins, sugar and cholesterol were tried to rear larvae of *H. axyridis*. These diets succeeded in their rearing until the 3rd instar but larvae never developed to the 4th instar on these CDs. Although many combinations of components and addition of some substances known to be effective for other insects were tried, no notable improvement was resulted. Consequently, these CDs lack some important factor(s) for *H. axyridis* growth. Drone honey bee brood powder (DP) is known to be the useful diet for many species of aphidophagous coccinellids. A water soluble fraction from defatted DP was effective on the larval development when it was added to the CDs at the ratio of 5% and 1st instar larvae developed to adults on this diet. The effective factor(s) was found to be substance(s) with low molecular weight (M.W. <10,000). In order to find active ingredients, this fraction was further fractionated and rearing tests were carried out using the fortified CD. This fraction was divided into four fractions by gel filtration and the subdivided fractions were also effective with some deterioration. The nature and role of these active ingredients were estimated.

20-098

FACTORS INVOLVED IN IN VITRO REARING *MELITTOBIA DIGITATA*M. Cooperband, P. Fanti¹, S.B. Vinson, R. W. Matthews²

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The ectoparasitoid *Melittobia digitata* (Hymenoptera: Eulophidae) can be reared in the laboratory using artificial media free of insect tissue. There are two other factors important to rearing. One is obtaining oviposition on the artificial host containing the diet and the other is providing a suitable membrane to allow access to the media. We evaluated several membranes which provide access to the food while reducing contamination of the media by the parasitoids. Oviposition onto the artificial host has been obtained and some of the factors that influence the female's response are discussed.

20-099

A SUBSTRATE FOR MEASURING FECUNDITY ON MIRID BUGS

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Mirid bugs are polyphagous predators used in biological control of greenhouse pests, including whiteflies and thrips. Routine test of fecundity in mass reared predators has to be done in order to check their quality as beneficials. Mirid bug eggs are difficult to see since they are completely inserted into the plant veins, petioles and stems. As their hosts are hairy plants, the difficult is even greater. Here we report on an easy way for measuring fecundity of mirid bugs. The oviposition substrate is a tobacco, tomato or cucumber leaf, with their main veins removed, placed on top of a thin agar layer (<4 mm). Bugs are fed with *Ephestia kuehniella* eggs. Mirid bug females oviposit in the leaf tissue and the whole egg is visible from the bottom of the cage, as cage and agar are transparent. Mean fecundity in leaf agar cages was not significantly different from fecundity on plant for the three plant species tested.

20-100

IN VITRO REARING OF *OOENCYRTUS KUVANAE* H. (HYMENOPTERA : ENCYRTIDAE) AND ITS OVIPOSITION BEHAVIOR

H. P. Lee, K. S. Lee, D. Y. Son

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In insectary condition (Temp. 27 ± 1 °C, RH $70 \pm 5\%$, L:D 16:8), *in vitro* rearing of *Ooencyrtus kuvanae*, parasitoid for Gypsy moth egg successfully achieved on artificial egg medium which used for *Trichogramma* sp..

For a effective inducing of the wasp's host acceptance on artificial egg bag-form, the kairomone of Gypsy moth egg extracted with ethanol treated on the surface of bag-form. The kairomone treated egg bag-form showed 5 times as high parasitism as untreated artificial egg bag-form, otherwise large number of females (40 females per artificial egg bag-form) were required to get above 90% parasitism for the one day exposure.

Adult female wasp reared from *in vitro* showed the greatest oviposition activities at 7 to 8 days after emergence. The period from egg to adult emergence were 15 ± 2 days and 10 ± 1 days for larval period, respectively. Most of the wasp were emerged in 15th to 16th day after oviposition and larval size of all instar were likely to be larger than those reared *in vivo*.

The first generation of wasp reared *in vitro* showed no difference in parasitism on natural eggs compared with the wasp reared *in vivo*.

20-101

A METHOD FOR MASS-REARING OF SPECIES *PODISUS MACULIVENTRIS* (SAY) (HETEROPTERA : PENTATOMIDAE)T. MANOLE, M. IAMANDEI¹

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The researches carried out in the period '90-'94 had allowed of the mass-rearing, for the first time in Romania, of the predator species *Podisus maculiventris* (Say).

In the paper the results concerning the feeding behaviour, the duration of larval development, the female fecundity, the eggs fertility, the adults longevity and the active period of female under controlled conditions are presented.

20-102

The species of *Trichogramma* Westwood in Iran

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Nine species of *Trichogramma* have been collected from different parts of Iran on various lepidopterous eggs. These species and their hosts are as follows:

Trichogramma evanescens Westwood on eggs of *Cydia pomonella* and *Chilo suppressalis*; *T. rhenana* Voegle & Russo on eggs of *Naranga aenescens*; *T. embryophagum* Hart. on eggs of *Archips rosanus*, *Cydia pomonella*, *Ocnerogya amanda* and *Spectrobates ceratoniae*; *T. cacoeciae* Marchal on eggs of *Cydia pomonella* and *Spectrobates ceratoniae*; *T. maidis* P.V. on eggs of *Chilo suppressalis* and *Ostrinia nubilalis*; *T. cordubensis* Vargas & Cabello on eggs of *Chilo suppressalis* and *Ostrinia nubilalis*; *T. semblidis* Auriv. on eggs of *Sepedon spegea*; *T. brassicae* Bezdenko on eggs of *Chilo suppressalis*; *T. pinto* Voegle, host unknown. Investigations are now being carried out on electrophoretic analysis of species and strains.

20-104

VARIATIONS IN PINE PROCESSIONARY CATERPILLAR EGG PARASITISM PRODUCED BY AUGMENTATION WITH LABORATORY REARED *OOENCYRTUS PITYOCAMPAE* (MERCET)R. Tiberi⁽¹⁾, T. Niccoli⁽¹⁾, A. Niccoli⁽²⁾

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In July 1993, a biological control trial was carried out to control the Pine Processionary Caterpillar, *Thaumetopoea pityocampa* (Denis et Schiffmüller) through the use of one of its egg parasitoids, *Ooencyrtus pityocampae* (Mercet), which was reared in the laboratory on *Nezara viridula* (L.) (Rhynchota, Pentatomidae) eggs. More than 26,000 individuals of the parasitoid were released in 7 different times over a period of six weeks into a pine stand of the *nigra* group in central Italy (Tuscany), in order to cover the whole flight period of the pine processionary moth. The following year, the percentage of host eggs that were parasitized by *O. pityocampae* increased more than double, from 7.3% to 17.7% and *O. pityocampae* became the most effective egg parasitoid. However, during the following two years, parasitism by *O. pityocampae* decreased considerably to only 5.53% in 1994 and to 6.31% in 1995. In spite of that, this Encyrtid remained the most effective among the egg parasitoids limiting *T. pityocampa* populations in this area.

20-103

FACTORS EFFECTING THE EFFICIENCY OF TRICHOGRAMMA COLONIZATION.

J. KOT

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Biological control has been carried out in Poland since 1958, using species of *Trichogramma* Westw. The factors which have been shown by empirical investigation to be capable of exerting a decisive influence on the effectiveness of *Trichogramma* are:

1. Species and ecotypes.
 2. Quality of *Trichogramma* adults.
 3. Type of host.
 4. Numbers and time of parasitoid releases.
 5. Biocenotic relations.
 6. Climate and microclimate.
- Size of parasitoids, longevity and number of eggs laid, ratio females to males, resistance to variability in temperature and humidity, resistance to insecticides used and the searching ability.
- Attractiveness of the target host eggs and their duration.
- Numbers of parasitoids in relation to the plant surface, the coincidence of host appearance and the time of parasitoid releases.
- Density of host eggs and predation of parasitised eggs.
- All the factors mentioned above never occur simultaneously, but there are frequent instances when some overlap, making it difficult to analyse them.

20-105

SOME RECORDS ON THE *TRICHOGRAMMA* SPECIES ASSOCIATED WITH *DIATRAEA* IN THE NEW WORLD (HYMENOPTERA: TRICHOGRAMMATIDAE - LEPIDOPTERA: PYRALIDAE).R.A. Zucchi, J.D. Pinto¹, R.C. Monteiro

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Among the *Diatraea* species found on sugar cane, *D. saccharalis* is the most common in several countries of the American continent. Therefore, in many of these countries, biological control programs are being carried out to control the sugar cane borer by releasing *Trichogramma*. However, in some cases, the specific names of *Trichogramma* have been used improperly and, there are no voucher specimens to verify the species identifications used in these programs. Consequently, a survey of records exclusively from the literature does not provide reliable information on the hosts and distribution of *Trichogramma*. The results of our studies are based on *Trichogramma* samples received for identification occasionally. Eleven species of *Trichogramma* from eggs of four *Diatraea* species from seven countries were identified as follows: USA- *T. exiguum*, *T. minutum* and *T. thalense* from *D. grandiosella* on corn. Mexico- *T. atopovirilia* on *D. grandiosella* and *D. considerata*, *T. exiguum* from *Diatraea* sp. and *T. fasciatum* from *D. saccharalis* (?). Dominican Republic- *T. fuentesi* from *D. saccharalis* on sugar cane. Brazil- *T. dissimilis*, *T. distinctum*, *T. galloi*, *T. jalmirezi* and *T. pretiosum* from *D. saccharalis* on sugar cane. Bolivia- *T. galloi* from *D. saccharalis* and *D. rufescens* on sugar cane, Paraguay- *T. galloi* from *D. saccharalis* on sugar cane, Uruguay- *T. galloi* from *D. saccharalis* on sugar cane; *T. pretiosum* from *D. saccharalis* on rice and sugar cane.

20-106

INDUCTION OF ADULT DIAPAUSE AND OVERWINTERING IN A PARASITOID WASP, *OOENCYRTUS NEZARAE*

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Ooencyrtus nezarae Ishii (Hymenoptera: Encyrtidae) is an egg parasitoid of true bugs. First, we reared *O. nezarae* on eggs of the bean bug, *Riptortus clavatus* Thunberg (Heteroptera: Alydidae) under various photoperiods and temperatures in the laboratory. Under short-day (10L-14D) conditions at 15 °C, all female adults entered diapause without vitellogenesis. Under long-day conditions at 20 °C, some adult females entered diapause. Then we kept eggs of *R. clavatus* parasitized by *O. nezarae* under conditions of natural photoperiod and temperature in Osaka, Japan. Diapause female adults emerged first in late October, and most female adults emerging in November entered diapause. The greater proportion of them overwintered, although no males did. Thus, only female adults of *O. nezarae* enter diapause under short-day conditions and low temperature and overwinter.

20-108

SELECTION OF A BIOLOGICAL CONTROL AGENT: HOST-ACCEPTANCE AND HOST PREFERENCE OF TWO SPECIES OF TRICHOGRAMMATIDAE

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Species of egg parasitoids of the Trichogrammatidae family are currently selected for the biological control of Lepidopterous pests (*Artogeia rapae*, *Trichoplusia ni* and *Plutella xylostella*) of cruciferous crops. Host acceptance and host preference of the three pests were measured for *Trichogramma buesi* and *Trichogrammatoidea bactrae*.

In no-choice experiments, host acceptance (proportion of females parasitizing at least one egg) by *T. buesi* was rapid for *T. ni*, 90% after 2 hours, and gradual for the two other hosts, ca. 50% after 2 hours and above 95% after 24 hours. *A. rapae* eggs were rejected by *T. bactrae* but acceptance of *P. xylostella* and *T. ni* was rapid, over 75% after 2 hours.

During a 4-hours choice experiment, *T. buesi* displayed a significant preference for *T. ni* and rejection of the two other hosts. *T. bactrae* preferred *T. ni*, rejected *A. rapae* and parasitized *P. xylostella* proportionally to its abundance. Video image analysis of a 20-minutes choice experiment gave the same results for *T. bactrae*. However, *T. buesi* displayed no significant host preference, *T. ni* and *P. xylostella* had similar acceptance/contact ratios and were parasitized proportionally to their relative abundance. *T. buesi* could show learning and be a more generalist parasitoid than *T. bactrae*.

20-107

FECUNDITY AND LONGEVITY OF *OOENCYRTUS PITYOCAMPAE* (MERCET) REARED ON EGGS OF A LABORATORY HOST, *NEZARA VIRIDULA* (L.)R. Tiberi⁽¹⁾, A. Niccoli⁽²⁾, P. Sacchetti⁽¹⁾⁽¹⁾ Istituto di Patologia e Zoologia forestale e agraria, Università di Firenze, Italy - ⁽²⁾ Istituto Sperimentale per la Zoologia Agraria, Firenze, Italy

Among the egg parasitoids of the Pine Processionary Caterpillar, *Thaumetopoea pityocampa* (Denis et Schiffmüller) (Lepidoptera, Thaumetopoeidae), only the Encyrtid *Ooencyrtus pityocampae* (Mercet) (Hymenoptera, Encyrtidae) was reared successfully in the laboratory on alternative hosts, especially Lepidoptera and Heteroptera. Among these, the Pentatomid *Nezara viridula* (L.) proved to be particularly suitable for the mass rearing of the parasitoid which are to be used in biological control programmes.

A wild strain of *O. pityocampae* from PPC egg masses was used to establish the laboratory rearing. Fecundity of *O. pityocampae* on the laboratory host increased from 50 eggs per female in the 1st generation developed on *Nezara* to 119 eggs after 28 generations. The average number of individuals that originated from each female increased from approximately 40 (in the 1st generation) to 93 after 28 reproductive cycles on the same host. The percentage of *O. pityocampae* females that accepted *N. viridula* eggs increased from 78.94% in the 1st generation to over 85% and reached 100% in the last generation tested. The females of the various generations began to oviposit in different moments of their live, which varied from a few to several days after emergence. Furthermore, the duration of the oviposition period varied greatly. The presence of two or three parasitoid females on the same egg mass did not seem to alter their reproductive capacity in any significant way. The longevity of the individuals varied from a minimum of 5 days (checked during the 6th generation) to a maximum of 63 days (during the 29th generation). On average, the most long-lived showed to be the females of the 12th generation (45 days).

20-109

RELEASE OF *TRICHOGRAMMA BRASSICAE* BEZDENKO ON GRAIN CORN FOR THE BIOLOGICAL CONTROL OF EUROPEAN CORN BORER, *OSTRINIA NUBILALIS* (HÜBNER), IN NORTH-EASTERN ITALY

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During the years 1991-95 research on the biological control of the European corn borer (ECB), *Ostrinia nubilalis* (Hübner), with the egg parasitoid *Trichogramma brassicae* (Bezdenko) was carried out in north-eastern Italy. About 200 000 parasitoids per hectare were released on grain corn three times against the ECB second generation using cardboard capsules containing parasitized eggs of *Ephestia kuehniella* Zeller; the first release occurred at the beginning of the ECB oviposition period (end of July), while the second and third ones followed at 7-10 day intervals. Parasitoids produced at Biolab (Cesena, Italy) or at Biotop (Valbonne, France) were used.

In treated fields the ECB egg masses parasitization ranged from 25% to 90% and was always higher than in untreated ones; the activity of naturally occurring trichogrammatids was delayed and rarely exceeded 20%. In fields where the parasitoids were released at 200 points per hectare the parasitization rate (45%) was higher than in those where *T. brassicae* was distributed at 100 points/ha (26%). The parasitization rate reduced rapidly from the release points and was very low (<15%) at a distance of over 10 m; moreover the parasitoids shifted downwind. A local strain of *T. brassicae* was constantly and significantly more efficient (average parasitization 74%) than a French strain (50%). When the oviposition period of ECB continued during to the first decade of September a 50-90% egg masses parasitization was observed up to 4 weeks after the third release.

Even though the parasitization rates observed were high, a considerable reduction in the number of ECB larvae and an increase in crop yield were not always observed at the harvest. Further research should be carried out before applying this biological method extensively in northern Italy.

If invited speaker, indicate title of session and name(s) of organizer(s):

20-110

ANAGRUS FENNICUS SOYKA (HYMENOPTERA: MYMARIDAE), EGG PARASITOID OF *CICADELLA VIRIDIS* (L.) (HOMOPTERA: CICADELLIDAE) IN ITALY

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Anagrus fennicus (Hymenoptera: Mymaridae), introduced by Soyka in his key of *Anagrus* females (1955) as a new species, together with 33 others, was never described in detail.

In the review of the European species of the genus (Chiappini, 1989), I kept this species as valid and added some distinguishing characters that I deduced from study of the holotype.

A. fennicus holotype was collected in Finland (28° lat., 68°10' long.), on 29-VIII-1938 and, up to now was the only specimen of the species available. No information existed on biology and hosts.

Thus, it is here recorded for the first time in Italy on a known host.

The specimens utilized for this study were reared from *Cicadella viridis* (L.) overwintering eggs deposited at the base of *Carex riparia* Curtis (Cyperaceae) leaves, collected periodically during the winter months, from September to February, in uncultivated areas along the Po river in Piacenza province in Italy.

A morphological description of *A. fennicus* is given, together with biological, ecological and ethological observations and, on these bases, its specific identity is discussed.

20-112

HOST NUMBER AND SPATIAL DISTRIBUTION INFLUENCE ON *TRICHOGRAMMA* (HYMENOPTERA) HOST ACCEPTANCE AND FECUNDITY

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Experiments with different *Trichogramma* species show that the percentage of females which accept *Sitotroga cerealella* Oliv. (Lepidoptera, Gelechiidae) for parasitization depends significantly on the number and spatial distribution of host eggs. The rest of *Trichogramma* females that refused *Sitotroga* eggs have many mature ovarian eggs, sometimes contact the host, but their parasitization behavior is interrupted at the stage of arrestment and host recognition.

In different *Trichogramma* species, over the range of the number of host eggs from 5 to 90, the percentage of parasitizing females increases by two to ten times. Females placed in Petri dishes 9 cm in diameter accept *Sitotroga* eggs more often than females placed in Petri dishes 4 cm in diameter, all other factors being the same. One artificial patch consisting of 60 host eggs provokes parasitization in significantly lower percentage of wasps than 12 patches consisting of 5 eggs each. In some cases the spacing between patches also influences the acceptability of host eggs. The mechanisms underlying the observed effects are not clear yet.

An increase in the number of host eggs may possibly result in higher concentration of kairomone which arrests the parasitoid and starts parasitization. Spatial distribution of eggs may influence host acceptance through the kairomone concentration as well. However, both visual and tactile perception may also participate in *Trichogramma* females reaction to the number and spatial distribution of host eggs.

20-111

INFLUENCE OF PARASITISM SURFACE POSITION ON *TRICHOGRAMMA CORDUBENSIS* (HYMENOPTERA: TRICHOGRAMMATIDAE) HOST FINDING ABILITY

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The oophagous parasitoids of the genus *Trichogramma* are used worldwide as biological control agents against several agricultural pests. The success of parasitism in the fields depends, amongst other factors, of the parasitoid host finding ability, which is related with the parasitism surface position. Therefore this study was concerned with *T. cordubensis* parasitism distributions over several surface parasitism levels, and inner or upper parasitism surfaces. For this species, the parasitism significantly increased with the increasing height of the parasitism surface position: an average of 9.6 parasitized eggs for the inner and upper surfaces of the egg card placed at 14 cm, 33 at 28 cm, 55.8 at 42 cm, 72.6 at 56 cm and 139 at 70 cm. For each parasitism surface level, the average of parasitized eggs per egg card was always higher at the inner parasitism surfaces, although significant differences were only observed for the egg cards placed at 42 and 56 cm of height.

20-113

DNA POLYMORPHISM ANALYSIS OF *TRICHOGRAMMA* (HYMENOPTERA: TRICHOGRAMMATIDAE) IN JAPAN

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For the quality control of *Trichogramma* as a biological agent, an efficient system to maintain strains is first thing to be established. In the present study, we evaluated reliability of polymorphisms in an internal transcribed spacer (ITS2) of ribosomal DNA and cytochrome oxidase III gene (COIII) of mitochondrial DNA as probes for different species and strains of *Trichogramma*. Nucleotide sequences were determined for ITS2 and COIII of several species and strains of *Trichogramma* collected in Japan. Both ITS2 and COIII showed sequences specific to each species examined, except *T. chilonis*. Thus, a possibility was shown to use gene polymorphisms as probes to identify strains of *Trichogramma*.

20-114

SUPERPARASITISM BY A NAIVE EGG PARASITOID, *TRICHOGRAMMA CHILONIS* ISHII (HYMENOPTERA : TRICHOGRAMMATIDAE) : EFFECT OF ELAPSED TIME AFTER FIRST OVIPOSITION BY CONSPECIFICS ON HOST DISCRIMINATION

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This study was carried out to investigate whether females of *Trichogramma chilonis* Ishii which had never oviposited in any host egg after emergence could discriminate between unparasitized and parasitized hosts by conspecific females or not. Mediterranean flour moth, *Ephestia kuehniella* (Zeller) eggs containing an immature parasitoid of either 0, 1, 2, 3, 5, 7 or 9 ages in days or eggs containing nothing (i.e. unparasitized eggs) were offered to *T.chilonis* females. Although the female parasitoids oviposited in unparasitized and 0 day parasitized eggs, they did only in a part of eggs of 1 and 2 days and avoided all the eggs more than 3 days. Moreover, two parasitoids were allowed to oviposit in the same host egg with an interval within 10 minutes or of 24 hours. Emerging rate of second offsprings from superparasitized hosts with time interval of less than 10 minutes was as much as first ones. However, that of 24 hours interval decreased down 20%. These results suggested that inexperienced females of *T.chilonis* could distinguish unparasitized and parasitized host eggs, and might adaptively oviposit in parasitized hosts according to survival of their offsprings.

20-116

A STUDY ON USING *TRICHOGRAMMA DENDROLIMI* TO CONTROL *MICROMELALOPHA TROGLODYTA* GRAESER

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Micromelalopha troglodyta is extensively dispersed over northeast China, north China, northwest China and east China. Its host plants include poplar, elm, birch and willow. They mainly endanger poplar in the wind-break forest of west Jilin Province. All leaves of the host plants were eaten up while they broke out, seriously affecting the growth and development of the trees and causing their withering in winter. *Trichogramma dendrolimi* was used to control *M. troglodyta* in a Station in Jilin Province in 1988. The experiment was carried out as follows: (1) Unfertilized eggs of *Antheraea pernyi* were used to propagate *T. dendrolimi* for 8 generations. (2) The reared parasitoids were released from 3rd-17th in June when the moths were laying eggs. (3) During the experiment, 20000 *T. dendrolimi* were released per mu, and the release was repeated for 4 times so that a total of 80000 *T. dendrolimi* per mu were released. (4) The parasitoids were released at a height of 2 meters above the ground, and the control spot was located windward away 1000 meters. The parasitic rate of the eggs ranged from 65-100%, 82% on average. The population of *M. troglodyta* declined 44% lower than that of the control spot.

20-115

EGG PARASITOID OF *PHORACANTHA SEMIPUNCTATA* F. (COLEOPTERA: CERAMBYCIDAE) IN SOUTHERN PORTUGAL. 1- FIELD STUDIES OF THE EFFICIENCY OF *AVETIANELLA LONGOI* SISCARO (HYMENOPTERA: ENCYRTIDAE)

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The eucalyptus longhorned borer *Phoracantha semipunctata* (Coleoptera: Cerambycidae), is a major pest causing serious damage in portuguese eucalyptus stands. Since 1992, the presence in Portugal of *Avetianella longoi* Siscaro (Hymenoptera: Encyrtidae), an egg parasitoid of *P. semipunctata*, has contributed to the reduction of the population of this insect. It may be useful, to employ this parasitoid in an Integrated Pest Management Program.

However, there has been little information on their effectiveness as a parasite.

The purpose of this investigation is to evaluate the effectiveness of *A. longoi* as a biological control agent in the field. During the summer of 1994 and 1995, every weeks, were collected egg layings of *P. semipunctata* in attacked trees.

Preliminary results of parasitized eggs, emerged parasitoids and sex-ratio were determined

20-117

TRACER DETERMINE SPREAD DISTANCE OF *TRICHOGRAMMA* WASPS REARED IN ARTIFICIAL HOST EGGS USING ³²P MARK IN THE CORN FIELD

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Trichogramma wasps reared in artificial host eggs were marked by ³²P in honey water in 16 hours, 10000 wasps of each species of *Trichogramma* were released in the corn field in each treatment. To investigate spread trace, fresh corn borer eggs oviposited in laboratory were placed at 10 m intervals from release wasp point (as the center of a circle) to all around. The results showed that 60% wasps of *T. chilonis* (in artificial eggs) could fly to 30 m from the center of circle, 20% to 50 m, 10% to 60 m and 10% to 70 m; 62.5% wasps of *T. dendrolimi* (in artificial eggs) could fly to 40 m, 12.5% to 60 m, 12.5% to 80 m, 12.5% to 90 m. These results showed no different from *T. dendrolimi* wasps (in *Antheraea pernyi* eggs). It indicated that artificial host egg could rear normal *Trichogramma* wasps.

20-118

A FIELD TRIAL OF USING *TRICHOGRAMMA CHILONIS* REARED IN ARTIFICIAL EGGS TO CONTROL *HELICOVERPA ARMIGERA*

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A field trial was carried out using *T. chilonis* reared in artificial eggs to control *Helicoverpa armigera* in the cotton field, Hebei province in 1994. There releases were made at the second generation egg stage, 351000 wasps per hectare. The result showed that the parasitising rate was 46.4%, the natural parasitising rate 0. There were five releases at the third generation egg stage, 1530000 wasps per hectare, parasitising rate 58.4%, the natural parasitising rate also 0.

T. chilonis reared in *Antheraea pernyi* egg was as a control. There were four releases at the egg stage of the third generation, 1125000 wasps per hectare. The parasitising rate was 74.6%, higher than that in the artificial egg wasp areas. When *T. cilonis* reared in *A. pernyi* was released at egg stage of the second generation, the parasitising rate was 67.8% at normal years. The main reason of lower parasitising rate this year was abnormal climate in which temperature was much higher and humidity was much lower than usual. There were 27 days in which temperature was above 30°C; 16 days above 33°C; 11 days above 35°C; the lowest humidity was 3.2%. The upper limit of development temperature of *T. chilonis* was 33°C at laboratory. *T. chilonis* released in the field could only survive 16hrs.

This trial was arranged at the area in which *H. armigera* has higher resistance to agricultural chemicals. The control efficiency in the wasp-releasing areas (*Trichogramma* of artificial egg and natural egg) were higher than that in chemical control area (eight spraying, 24 bollworms on one hundred plants). The mortalities of *H. armigera* were 58.3% and 83.3% in the wasp-releasing areas, the percentages of borehole cotton buds and bolls were decreased to 80.8% and 84.6% respectively, the number of natural enemies (ladybirds and lacewings) was doubled.

20-120

NON - KILLING EFFECTS OF CERTAIN INSECTICIDES ON THE DEVELOPMENT AND PARASITIC FEATURES OF *TRICHOGRAMMA*B. Ramesh¹ and P. Baskaran²

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The effect of seven insecticides recommended for the control of several lepidopteran pests was studied under laboratory conditions on adult parasitic features and development of *Trichogramma*. The concentration of the insecticides are those recommended for field application viz., Endosulfan 0.0175%, Monocrotophos 0.09%, Phosphamidon 0.09%, Cypermethrin 0.016%, Achook 0.5%, Dipel 0.004%, and Nimbecidine 0.002%. Biopesticides Achook, Nimbecidine and Dipel were found to be completely safer both to adult parasitic features and developmental stages of *Trichogramma*. Among insecticides, Endosulfan was comparatively safer to the parasitoids, while Monocrotophos and Phosphamidon although had inhibitory effects were better than Cypermethrin which was found to be highly lethal to the parasitoids.

20-119

DOES THE HOST AVAILABILITY REGULATE THE LONGEVITY AND FECUNDITY OF *TRICHOGRAMMA* ?B. Ramesh¹ and P. Baskaran²

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Biological hypothesis that the female parasitoids could adjust their fecundity schedule according to host availability and tradeoff longevity with fecundity was tested in a thelytokous egg parasitoid *Trichogramma brasiliensis* Ashmead. The tradeoffs between early and late reproduction revealed that the test insect was able to exploit its hosts as much as possible during the first day or first few days of adult life. During periods of no host, it was able to adjust its fecundity schedule and compensate early reduction in the later stages. Secondly, in tradeoffs between longevity and fecundity there existed a negative relationship between survival and reproduction i.e. oviposited females lived shorter. From these two observations it appears that *T. brasiliensis* responded positively to the hypothesis and hence their suitability under fluctuating host availability in inundative releases can be recommended to achieve effective control.

20-121

IMPACT OF PLANT CHARACTERS ON *TRICHOGRAMMA* SEARCHING BEHAVIOR: A CASE STUDY WITH PIGEONPEA *CAJANUS CAJAN*J. Romeis, T. G. Shanower, C. P. W. Zebitz¹

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Trichogramma spp. only rarely parasitizes eggs of *Helicoverpa armigera* (Hübner) on pigeonpea, while on other plants high parasitism levels of this host are found. The reasons for the obvious rejection of pigeonpea were studied in the laboratory and in the field in a pigeonpea:sorghum intercrop.

Olfactometer studies have shown that odors emitted by pigeonpea plants in the reproductive stage, the growth stage preferred by *H. armigera* for oviposition, repel the searching parasitoids so that landing on the plants may be avoided. In comparison, the odor of flowering sorghum panicles, a plant from which high parasitism levels are reported, were attractive to the parasitoids.

After landing on the pigeonpea plant three other factors mediate the searching behavior of the parasitoids: (1) As shown in a filterpaper bioassay, hexane surface washings from pods and leaves contain contact repellent(s); Walking speed on leaves increased significantly after washing with hexane, (2) glandular and nonglandular trichomes on the reproductive plant structures inhibit walking behavior, and (3) the parasitoids are trapped by the sticky exudates secreted by glandular trichomes. The latter factor seems not to be an important parasitoid mortality factor in the field.

In a pigeonpea:sorghum intercrop in the field, the high *Trichogramma* population in sorghum did not move over into the pigeonpea crop where a very low parasitoid density was observed throughout the season.

20-122

PRIMING *APHYTIS*: BEHAVIORAL MODIFICATION OF HOST SELECTION BY EXPOSURE TO A SYNTHETIC CONTACT KAIROMONE

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The introduced parasitic wasp, *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), utilizes a kairomone, *O*-caffeoyltyrosine (*OCT*), to recognize California red scale, *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae). Wasps used in augmentative release programs for California red scale on California citrus are reared on oleander scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae), themselves reared on squash. The goals of this study were: 1) to determine if long-term rearing on oleander scale caused *A. melinus* to develop a preference for oleander scale, 2) to determine if the preference of oleander-reared *A. melinus* for California red scale might be enhanced by exposing them to synthetic *OCT* in the laboratory, and, if so, 3) to determine if oleander-reared but *OCT*-primed wasps parasitized a greater proportion of California red scale in the field than unconditioned wasps.

Wasps that emerged as adults isolated from their hosts retained a strong preference for California red scale regardless of rearing host. This preference was reduced if wasps were allowed to emerge from oleander scale, thus acquiring early adult experience with oleander scale. The preference for California red scale was restored, however, by exposing wasps reared on oleander scale to synthetic *OCT*.

Preliminary field results suggest that wasps primed to *OCT* parasitize more California red scale than control wasps. Exposure of *A. melinus* reared in commercial insectaries to *OCT* prior to release may be a means to improve the effectiveness of augmentative release programs to control California red scale.

20-124

Response of *Chrysopa septempunctata* to pheromones of aphidsK.S. Boo, I.B. Chung¹, L.J. Wadhams, J.A. Pickett²
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Sex pheromone, (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, and alarm pheromone, (E)- β -farnesene, of aphids were tested for their effects on *Chrysopa septempunctata*, one of the principal predator of aphids. In EAG tests, both of sex pheromone components showed a responsiveness but alarm pheromone gave no difference from that of solvents. Sex pheromone components also showed an attractiveness in a Y-tube olfactometer and field trapings with a higher attractiveness of (4a*S*,7*S*,7a*R*)-nepetalactone but alarm pheromone showed no evidence for attraction in both of laboratory bioassay and field trapping. Interestingly, spring and summer populations of *C. septempunctata* gave no response to sex pheromone components either.

20-123

PREDATORY MITES CEASE TO RESPOND TO PLANT VOLATILES: A CONTAGIOUS PHENOMENON

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During foraging for spider-mite prey, the predatory mite *Phytoseiulus persimilis* is highly attracted to plant volatiles that are produced in response to spider-mite feeding. This phenomenon has been recorded consistently for several predator populations during more than 10 years. During a recent 5-year monitoring period, we have recorded for two of these populations that the predators lost their attraction to prey-infested plants.

The inability to respond to plant volatiles is accompanied by several other phenomena such as: high crystal load in the Malpighian tubules, reduced reproduction, reduced size, increased mortality.

The cause of this syndrome appears to be a non-genetical change in the predators that is contagious. When a predator from a 'normal' population is brought together with an individual from a non-responding population, the phenomena of the latter predator can be found in the 'normal' predator within a few days.

20-125

MEDIATION OF HOST LOCATION BY VOLATILES IN THE GRAIN WEEVIL PARASITOID *LARIOPHAGUS DISTINGUENDUS* (FÖRST.) (HYMENOPTERA: PTEROMALIDAE)

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The role of volatiles for host location in parasitoids was examined in a tritrophic system, consisting of *Lariophagus distinguendus* (Först.), a parasitoid of different stored product beetles, the grain weevil *Sitophilus granarius* (L.), a worldwide pest which develops in kernels of stored grain, and kernels of wheat (*Triticum* sp.).

The experiments were performed in a four chamber olfactometer, at first using female parasitoids with oviposition experience. The parasitoids were attracted by volatiles emanating from uninfested and infested wheat kernels. They were able to differentiate between infested and uninfested kernels with infested kernels being more attractive. The potential volatile sources of an infested wheat kernel are weevil larvae, their frass and the damaged kernel. From these, the frass turned out to be most attractive to the parasitoids followed by infested kernels from which the larvae and the frass had been removed. These kernels were as attractive as uninfested, but cutted kernels. This indicates that their attractivity could be due to the mechanical damage of the kernels by the feeding weevil larvae. The larvae had no significant attractive effect. Experiments with inexperienced female parasitoids which were kept isolated from uninfested and infested grain upon emergence, indicate an increase of the responses to frass, mechanically damaged kernels and uninfested kernels due to experience.

Thus *L. distinguendus* uses volatile cues from both trophic levels, host (frass) and host plant (grain and damaged grain) for host location and modulates the response to these cues by experience.

20-126

RESPONSES OF PARASITOIDS OF APHIDS TO OLFACTORY CUES OF THE HOST PLANT OF THEIR PREY

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A range of parasitoids of aphids from monocotyledonous and dicotyledonous plants, both trees and herbs, all reacted positively to the odour of the plant species on which they had developed, even when they emerged from mummies detached from plants. The odour of the plant was preferred to that of the prey aphid and its honeydew.

Such discrimination in favour of the plant on which the parasitoid had developed was even shown by *Aphidius rhopalosiphii* De Stef. between different cultivars of wheat. It is suggested that it is the balance of the common volatiles from wheat, rather than cultivar-specific characters, which underlies the discrimination.

If the parasitized aphids are transferred to a different wheat cultivar at various times after parasitisation, it can be shown that it is the cultivar experienced before pupation by the parasitoid which is preferred on emergence of the adult. This appears to vindicate Hopkins' 'host selection principle' proposed as long ago as 1917, that chemical experiences of the larva can be transferred to the adult through the pupal stage in an endopterygote insect. Previous evidence for Hopkins' principle has never been accepted as free of alternative interpretation and unfortunately this example of aphid parasitoids also turns out clearly not to be an example of the principle. However, preferences of parasitoids seem to be revealed which are normally masked by previous experience of host plants.

20-128

THE ROLE OF CHEMICALS FROM DAMAGED LEAF IN HOST FINDING BY PARASITIC WASPS

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Parasitoids use several chemicals of host food plant origin as cues of presence of host. The functions of such chemicals differ according to their volatility: parasitoids can detect volatile cues at a distance from a food plant whereas they can detect nonvolatile cues by contact chemoreceptor to confirm a host food-plant. In addition the damage by host feeding may induce a plant to produce some specific compounds. Such host-induced plant chemicals seem to be reliable cues for the parasitoids on the presence of hosts on the plant.

It is a matter of argument on the evolution of such chemical cues whether the plant has evolved to produce the chemicals or whether the parasitoid has evolved to detect the chemicals closely related with host presence. We will attempt to examine the hypothesis that the plant has evolved to produce some chemicals leading the parasitoid to host larvae in the following two systems.

(1) Crucifer plants, *Pieris rapae* larvae, *Cotesia glomerata*(2) Corn plants, *Pseudaletia separata* larvae, *Cotesia kariyai*

20-127

LEARNING OF PLANT CHEMICALS BY THE EGG-LARVAL PARASITOID, *ASCOGASTER RETICULATUS* (W.) (HYMENOPTERA: BRACONIDAE)

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Ascogaster reticulatus Watanabe is the egg-larval parasitoid of the smaller tea tortrix, *Adoxophyes* sp. (Lepidoptera: Tortricidae). The females find host eggs using host kairomones located in wing scales and the egg mass surface. We found that the females could learn plant chemicals associated with the host egg mass. We treated a line of the plant extract (EtOH) on the bottom of a petri dish (9cm in dia), and the host egg mass was put at the center. One female was released in the dish and her behavior was observed until she began to oviposit. One or two minutes after the onset of oviposition, the wasp was gently removed from the host egg mass and put back in a cage. This procedure (training) was repeated one to three times with one hour's interval. Next, the wasp was released in the dish only with the plant extract, and the wasp responded to the treated part with her antennae and walked along the line. We measured the distance of walking on the line within 3 min, which was the criteria to see the learning response of female wasps. Their response increased with a corresponding increase in training time. We tested leaves of two plant species, and the response was stronger in the tea leaves, the host plant of *Adoxophyes* sp. than mulberry, non-host plant. Once they learned of the plant chemical after 3 experiences, they responded to it after 24 hrs to a lesser degree, and did not respond at all after 48 hrs.

20-129

VIBRATIONAL INTERACTIONS BETWEEN A LEAFMINER AND ITS PARASITOID

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Parasitoids rely on visual, chemical, and mechanical stimuli for efficient host location. If the host is concealed, like with many leafminers, substrate vibrations triggered by the host or the parasitoid may play an important role for interactions between both opponents.

We focused on behavioural interactions between the parasitoid *Sympiesis sericeicornis*, Nees (Hym. Eulophidae) and the apple leafminer *Phyllonorycter malella*, Gcr. (Lep. Gracillariidae). At different locations on the leaf lamina vibrations were measured using a laser vibrometer and were characterised by temporal pattern, amplitudes, and frequencies¹. Vibrations triggered by a moving larva are distinguishable over the whole leaf from background noise, and are specific to host stage and behaviour. The behavioural interactions between host and parasitoid were investigated using combined ethograms. The moving behaviour of the larva inside the mine strongly affects some of the transition probabilities of the parasitoid's activity. However the host's movements did not lead to obvious vibrotaxis. On the other hand insertion of the parasitoid's ovipositor has a significant influence on larval behaviour². Thus, both opponents interact by means of vibrational information incidentally transmitted via substrate.

¹ Meyhöfer, R. ; Casas, J. ; Dorn, S. (1994): Host location by a parasitoid using leafminer vibrations: characterizing the vibrational signals produced by the leafmining host. *Physiological Entomology*, 19 (4).

² Meyhöfer, R. ; Casas, J. ; Dorn, S. (submitted): Interspecific vibrational communication in a leafminer parasitoid system.

20-130

Cues for oviposition choice in *Episyrphus balteatus* (Diptera: Syrphidae)

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Aphid predators have to cope with unstable food supply for their offsprings because aphid colony have currently a short life time and may decline before predator offsprings maturation (Hemptine et al, 1993). In this context, a gravid female of *E. balteatus* have to take the decision to lay eggs in an aphid colony and thus must estimate the risk for her progeny in terms of food availability and intraspecific competition.

Our aim was to determine the cues that guide her choice. We tested the presence of aphid and their density; the presence of honeydew; also the presence of eggs or larvae of other individuals and the possibility of marking the oviposition site.

It appears that two kinds of chemical cues help Syrphid in their choice: the aphid odour enhancing oviposition and Syrphid's eggs and larva inhibiting it.

Reference: Hemptinne J. L.; A. F. G., Dixon; J. L. Doucet & J. E. Petersen. 1993. Optimal foraging by hoverflies (Diptera: Syrphidae) and ladybirds (Coleoptera: Coccinellidae): Mechanism. Eur. J. Entomol. 90: 451-455.

20-131

RESPONSE OF *TRICHOGRAMMA EVANESCENS* TO SYNTHETIC (Z,E)-9,12-TETRA-DECADENYL ACETATE (TDA), A SEX PHEROMONE COMPONENT OF *EPHESTIA KUEHNIELLA* AND *PLODIA INTERPUNCTELLA* (HYMENOPTERA: TRICHOGRAMMATIDAE - LEPIDOPTERA: PYRALIDAE)

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In field trials to evaluate *T. evanescens* WESTWOOD for biological control of stored product pest moths, the egg parasitoids were found on delta traps containing TDA. The possible use of the moth sex pheromone as a kairomone by *T. evanescens* was therefore studied.

In a four-armed airflow olfactometer, the position of mated wasps was observed directly and recorded using computer software. For a period of ten minutes one airflow permeated with TDA was tested against three others containing clean air. The experiments were conducted with naive females, females with oviposition experience and females which had oviposited in the presence of TDA.

Females which had previously oviposited in the presence of TDA spent significantly more time walking in the odour field containing TDA compared to clean air. Such a response was not elicited in both naive females and females with oviposition experience.

It is suggested that the females responding to TDA learned to use the moth sex pheromone as a kairomone. Learning may be especially important for host location in generalist parasitoids which frequently oviposit in eggs belonging to host species which have not been previously encountered.

As naive females were released in the field trials and the number of *T. evanescens* in delta traps was low, there seemed to be little impact of the monitoring programme on the fate and the effectiveness *T. evanescens*.

20-132

HOST SEARCHING BEHAVIOR OF *SYMPIESIS STRIATIPES* (ASHMEAD) (HYMENOPTERA: EULOPHIDAE), A PARASITIC WASP OF THE CITRUS LEAF MINER *PHYLLOCNISTIS CITRELLA* STAINTON (LEPIDOPTERA: GRACILLARIDAE)

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Female parasitic wasps of *Sympiesis striatipes* (ASHMEAD) attack the larvae of the citrus leaf miner *Phyllocnistis citrella* STAINTON that infest young growing leaves of citrus trees. Host searching behavior by the females wasps on the unmined lamina and on the mine of a citrus tree *Citrus unshiu* M. c.v. SUGIYAMA was observed with a video camera under semi-natural conditions.

The observed paths of host searching behavior of the wasps were segmented based on a temporal sampling method (wasp's movement per unit time). Then the segmented paths, i.e.steps, were analyzed for the frequency distributions of turning angles and correlations between successive searching steps. The analyses results revealed that the wasps detect and locate the host using cues coming from the mine made by the host and the host itself rather than at random.

In addition, the observed paths of host searching behavior were segmented and analyzed using a spatial sampling method (wasp's movement per unit distance). Results of searching behavior analyzed with the spatial sampling method gave poorer information in comparison with those analyzed with the temporal sampling method.

The application advantage of the temporal sampling method to the analyses of host searching behavior by the wasp is discussed.

20-133

CAN PARASITOID FLIES REGULATE THEIR CLUTCH SIZE IN RESPONSE TO HOST DENSITY?

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Effect of host encounter rate and host density per patch on the oviposition decision of *Exorista japonica* (Diptera: Tachinidae) females was studied in the laboratory, and their ability of host discrimination was examined. The number of eggs laid per bout increased as the rate of host encounter decreased and as host density per patch increased. When an unparasitized and a parasitized host were simultaneously presented to a female for 60 min, females did not discriminate between an unparasitized and a parasitized host with 5 eggs, but did between an unparasitized and a parasitized host with 10 eggs. The rate of host encounter also affected the host discrimination. Females did not avoid oviposition even on a parasitized host with 10 eggs when they encountered hosts at the interval of 24 h, but they laid less eggs on the parasitized host at 1 min interval of the host encountering rate. The results thus indicated that *E. japonica* females had the ability not only to regulate clutch size with the host density (the rate of host encounter) but also to discriminate between parasitized and unparasitized hosts.

20-134

THE ROLE OF HOST-INDUCED PLANT SYNOOMONES ON IN-FLIGHT ORIENTATION OF THE APHID PARASITOID *APHIDIUS ERVI* HALIDAY (HYMENOPTERA, BRACONIDAE)
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The aphid parasitoid *Aphidius ervi* Haliday is attracted by volatiles released by broad bean (*Vicia faba*) plants infested by the pea aphid, *Acyrtosiphon pisum* (Harris). Previous studies demonstrated that the attractiveness of infested plants was not significantly reduced when they were tested soon after removing the aphids. To assess the origin of the involved volatiles the basal leaves of a broad bean plant were isolated and infested with mixed instars of *A. pisum*, and the apical part of the plant was subsequently used as an odour source in a wind tunnel bioassay. These experiments demonstrated that attractive plant volatiles are systemically induced by aphid feeding and suggested that the biosynthesis of these compounds is dependent by host-induced changes of plant metabolism. *A. ervi* females were also able to discriminate between pea aphid infested plants and those infested by the non-host *Aphis fabae*. The isolation and chemical analysis of volatiles from infested plants showed the occurrence of compounds which appeared to be attractive to *A. ervi* females. However, most of these compounds were also detectable in intact plants, even though aphid damaged plants showed a significant alteration of their synthetic rate and relative ratio.

20-136

THE PHORETIC EGG PARASITOID, *TELENOMUS EUPROCTIDIS* (HYMENOPTERA: SCELIONIDAE), USES SEX PHEROMONE OF THE TUSsock MOTH *EUPROCTIS TAIWANA* (LEPIDOPTERA: LYMANTRIIDAE) AS A KAIROMONE

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The phoretic egg parasitoid, *Telenomus euproctidis* (Hymenoptera: Scelionidae), was found more frequently on virgin than mated female, *Euproctis taiwana* (Lepidoptera: Lymantriidae), when virgin and mated moths were exposed concurrently in the field for 24 h. Synthetic component of the moth's sex pheromone *E. taiwana*, (Z)-16-methyl-9-heptadecenyl isobutyrate, attracted both the wasp, *T. euproctidis*, and male *E. taiwana*. These findings suggest that *T. euproctidis* uses the sex pheromone of the female moth, *E. taiwana*, as a kairomone to locate host female moth and through her the host eggs.

20-135

COLOUR VISION AND OVIPOSITION BEHAVIOUR IN *APHIDIUS ERVI* HALIDAY (HYMENOPTERA, BRACONIDAE)

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The host recognition and acceptance behaviour of the aphid parasitoid *Aphidius ervi* Haliday is regulated by both chemical and physical cues, and visual cues appear to play an important role (Battaglia *et al.*, 1995, J. Insect Behav., 8, 739-750). Capillary glass tubes sealed on the flame and filled with artificial colours were used to evaluate the oviposition reaction of *A. ervi* females. The highest rate of oviposition reaction was obtained in the yellow region. However, colorimetrically different yellows elicited different ovipositional responses, with a peak value of 95% of reacting females in the case of a chemically defined colour. This spectacular oviposition reaction to colour is being used to develop a technical device for obtaining parasitoid oviposition on artificial diets. However, in natural conditions the oviposition behaviour is also controlled by chemical cues. In fact, both aphid dummies coated with cornicle secretion of the pea aphid, *Acyrtosiphon pisum* (Harris), or exuviae of this host aphid stimulated parasitoid oviposition, which was also observed when the bioassay was carried out under monochromatic red light. This experimental condition, in contrast, totally suppressed the oviposition response on colour filled capillary tubes.

20-137

TELENOMUS BUSSEOLAE (HYMENOPTERA: SCELIONIDAE) EGG PARASITOID OF *SESAMIA NONAGRIODES* (LEPIDOPTERA: NOCTUIDAE) RESPONDES TO SYNTHETIC SEX PHEROMONE IN OLFACTOMETER

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Host olfactory stimuli are important cues during the host location process and the use of host pheromones offers potential in improving the effectiveness of insect parasitoids.

Telenomus busseolae Gahan is an egg parasitoid recently introduced in Italy from Turkey against corn borer *Sesamia nonagrioides* (Lefevre), a serious pest of gramineous crops in the Mediterranean region. The possibility that the synthetic sex pheromone blend has some effect on the host finding behaviour of *T. busseolae* has been investigated.

A four component (Z-11-16:Ac/Z-11-16:OH/Z-11-16:Ald/12:Ac; ratio of 8.5/1/1/2) synthetic sex pheromone blend of the corn borer, commercially available in Italy, has been tested in y-olfactometer as a whole and as single components. Components were used in one of the arms, while the other was the control. Single wasps were introduced into the common arm and observed for 10 min. The first arm entered by the female was scored, the number of visits per arm was counted and the time spent in each of them measured.

Wasps are strongly attracted by the whole blend. The arm containing the odour received a significantly higher number of first choices than the control. In addition the parasitoids spent considerably more time in the test arm.

20-138

ADAPTIVE FORAGING STRATEGIES IN HYMENOPTERAN PARASITES: THE RELATION BETWEEN PARASITOID ECOLOGY AND LEARNING.

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For insect parasitoids, learning during foraging is well documented. Host seeking females can learn olfactory and visual cues associated with their hosts or the hosts micro-habitat and experience can strongly affect preference for major foraging cues. Theory on the adaptive value of learning in foraging suggests that an animal foraging in a predictable homogeneous environment and/or making only a few foraging decisions is not expected to use learning in foraging. We studied the role of learning in host foraging in *Cotesia flavipes* (Hymenoptera: Braconidae), a parasitoid of stemborer larvae with an ecology where learning is expected to be of low adaptive value. Using experimental procedures similar to other parasitoid learning studies, we determined the role of the learning mechanisms priming and preference-induction in the foraging of *C. flavipes*. We did not find any evidence that *C. flavipes* uses odour learning in host-microhabitat location. There was no significant effect of the development and emergence environment on the response level or preference towards infested plant odours. Neither did we find evidence that experience with a particular plant-host-complex during foraging influences subsequent foraging decisions in *C. flavipes* females. The absence of learning in *C. flavipes*, which seems an exception among the parasitoids studied, is discussed in relation to its ecology.

20-140

THE POISON GLAND OF THE PARASITIC WASP *Opius concolor* (Hym., Braconidae) CONTAINS REO- AND CORONA-LIKE VIRUS PARTICLESJ.A. Jacas¹, F. Budia², E. Viñuela², E. Rodríguez³, A. Fernández⁴.

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Virus-Like particles (VLP) have been found in the poison glands of adult females of *Opius concolor*. The VLP's are found in the secretory cells either free in the cytoplasm or within cytoplasmic vesicles, sometimes associated to a secretory apparatus. Negative staining has revealed the occurrence of two different particles: the first type exhibits a typical morphology of Reovirus-Like particles with a double shelled icosahedral structure (outer diam. 70 nm, inner diam. 60 nm) and hollow surface spikes. The second type was pleomorphic (diameter 30-60 nm) and had an envelope with club-shaped projections as classical Coronaviruses. Function and full characterization of these particles are under way, but it is presumed that they may play a role in the regulation of the tephritid hosts of this wasp, therefore being mutualistic with the latter and parasitic with the flies. It is the first time VLP's isolated from venom glands of a parasitic wasp exhibit either Reo- or Corona-Like viral nature.

20-139

MALE ANTENNAL STRUCTURES OF SOME PARASITOID ACULEATA (HYMENOPTERA) POSSIBLY INVOLVED IN SEXUAL RECOGNITION.P.L. Scaramozzino, G. Pagliano¹, R. Antonelli², N. Isidoro³, F. Bin³.

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Scanning Electron Microscopy observations of male antennae of numerous species of parasitoid Aculeata belonging to several families (Sapygidae, Tiphidae, Mutillidae, Eumenidae, Pompilidae, Sphecidae) have shown the presence of modified cuticular areas, generally hairless, with peculiar sculpture and pores. These areas often correspond to "tyloids" that are considered to be sensory structures. The pores may be the opening of glandular canals, so that further investigation with Transmission Electron Microscopy are in progress to confirm such an hypothesis. These male cuticular areas are probably involved in the chemical stimulation of the female antennal sensilla during mating behaviour, as it happens in some species of parasitoid Parasitica showing similar structures.

The presence of this type of cuticular areas in male antennae of non parasitoid Aculeata, like Vespidae, Anthophoridae and Andrenidae, suggests this phenomenon may concern many more groups of Hymenoptera.

20-141

ANTENNAL SENSORY AND SECRETORY STRUCTURES OF *COPTERA OCCIDENTALIS* MUES. (HYMENOPTERA: DIAPRIIDAE)P. Sacchetti, A. Belcari, N. Isidoro¹, F. Bin¹

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Antennal structures of *Coptera occidentalis* Mues., a Nearctic pupal parasitoid of the walnut fruit fly *Rhagoletis completa* Cress., have been investigated by Scanning and Transmission Electron Microscopes.

Male and female antennal sensilla and glands have been mapped and described. Eight types of sensilla, belonging to aporous, uniporous and multiporous type, have been found.

Aporous sensilla include campaniform type present on the first antennomere or A1 (both sexes), hair plates (probably proprioceptors) located on the proximal and distal margin of A1 (both sexes), trichoid type (some of which tactile) distributed on all the antennomeres (both sexes) and coeloconicum type (probably thermo-hygroreceptor on the lateral side of the A7-A12 in female and on A6-A13 in male).

Uniporous sensilla include only chaeticum type (probably gustatory) present on the ventral side of the A7-A12 in female and arranged in two rings on the A3-A14 in male.

Multiporous sensilla consist of two differently sized sickle-shaped sensilla (probably olfactory) in all antennomeres (except A1-A2) of both sexes and short sensilla basiconica (probably gustatory) present only in female and arranged in patch on the ventral side of A7-A12. Male antennae show two ventral glands on A3 and A4 with different release structures consisting of a peg-like process (A3) and a porous carina (A4).

The possible roles of secretory and sensory structures in mating behaviour and host recognition are discussed.

20-142

COMPARATIVE EXPERIMENTS WITH THE PREDATORY BUGS *ORIU* *INSIDIOSUS*, *O. ALBIDIPENNIS* AND *O. LAEVIGATUS* (HEMPTERA: ANTHOCORIDAE) TO IDENTIFY THE BEST PREDATOR FOR BIOLOGICAL CONTROL OF THE WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) IN GLASSHOUSE SWEET PEPPERS (*CAPSICUM ANNUUM*).

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Two comparative laboratory experiments in which biological parameters of *O. insidiosus* and *O. albidipennis* and *O. albidipennis* and *O. laevigatus* were done, it was concluded that the fecundity of *O. albidipennis* was higher than those of *O. insidiosus*, especially in the beginning of the egg laying period, while the fecundity of *O. laevigatus* was significantly higher than that of *O. albidipennis*. The longevity and fecundity of *O. laevigatus* females were also significantly higher than those of *O. albidipennis* when lacking animal food. The laboratory experiments demonstrated *O. laevigatus* as the best predator for use in IPM programs in glasshouse sweet peppers.

Two comparative glasshouse experiments in commercial sweet peppers in which *O. insidiosus* and *O. albidipennis* and *O. albidipennis* and *O. laevigatus* were simultaneously released for the control of the western flower thrips, *F. occidentalis*, showed that *O. albidipennis* totally replaced *O. insidiosus* and that *O. laevigatus* totally replaced *O. albidipennis* during the experimental period. The glasshouse experiments confirm the earlier found laboratory results very nicely.

20-143

EFFECTS OF FOUR INSECTICIDES ON *ORIU* *LAEVIGATUS* (FIEBER) (HEMPTERA: ANTHOCORIDAE)

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The anthocorid bug *Orius laevigatus* (Fieber) is widely promoted as an effective biological control agent for the western flower thrips, *Frankliniella occidentalis* (Pergande), a major pest of greenhouse crops. The susceptibility of this predatory bug to the insect growth regulators diflubenzuron and pyriproxifen, the thiourea compound diafenthiuron and the nitroguanidine insecticide imidacloprid was investigated in the laboratory. Toxicity of these compounds by exposure to treated glass plates, by ingestion via treated drinking water and by feeding on surface-treated eggs of *Ephestia kuehniella* Zeller to fifth-instar nymphs and adults of *O. laevigatus* was assessed. Pyriproxifen did not affect the viability of nymphs nor adults. Diflubenzuron was harmless to adults, but toxic to nymphs by residual contact (LC₅₀: 391 mg a.i./l) or by ingestion via drinking water (LC₅₀: 230 mg a.i./l). Diafenthiuron was more toxic to adults (LC₅₀: 100 mg a.i./l) than to nymphs (LC₅₀: 329 mg a.i./l) by residual contact, but harmless by ingestion via drinking water and by feeding on treated *Ephestia*-eggs. Imidacloprid had adverse effects on the survival of nymphs and adults of the predator, whatever the method of exposure (e.g. LC₅₀: 0.65 mg a.i. /l for adults after residual contact). Preliminary results show that even systemically applied imidacloprid is highly toxic to *O. laevigatus* wandering on the leaf surface.

These results indicate that integrated pest management programmes involving diflubenzuron, diafenthiuron and imidacloprid should regard possible side-effects to this beneficial insect.

20-144

EFFECT OF SOME INSECT GROWTH REGULATORS ON THE PREDATORY BUGS *ORIU* *LAEVIGATUS* (FIEBER) (HEMPTERA: ANTHOCORIDAE)

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Laboratory trials were carried out in order to test the effectiveness of seven insect growth regulators (tebufenozide, fenoxycarb, esafumuron, buprofezin, alsystin, teflubenzuron and diflubenzuron) on *Orius laevigatus* (Fieber). The insecticides were administered by contact at concentrations recommended for agricultural use.

The results with regard to the number of living adults obtained from 4 th (20%) and 5 th (80%) instars larvae, after exposure to fresh spray pesticides deposit on glass Petri dishes for 7 days, showed that all products but for esafumuron had no appreciable effect. Esafumuron slightly affected the development of immature stages into adults (63.3% mortality Abbott).

This treatments did not have any sensible influence on the number and on the eggs fertility of females obtained from 4 th and 5 th instars of treated larvae.

When treated with the five inhibitors of chitin synthesis esafumuron, buprofezin, alsystin, teflubenzuron and diflubenzuron, none of the 1 st (50%) and 2 nd (50%) instar larvae, after exposure to fresh spray pesticides deposit, developed into adults.

The experimental trial proves that the contact activity of the juvenile hormone mimic fenoxycarb and the ecdysis hormone mimic tebufenozide is approximately that of the inhibitors of chitin synthesis used.

20-145

SUPER-COOLING POINT IN ADULTS OF *ORIU* *SAUTERI* AND *O. MINUTUS* (HEMPTERA: ANTHOCORIDAE).

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Orius species are known as important natural enemies attacking agricultural pests such as thrips, mites or aphids. *O. sauteri* and *O. minutus* are most common in Japan and they overwinter as adults. However, only females can survive until the next spring. Males are likely to have low cold hardiness. Since the overwintered females are already mated, they are able to lay fertile eggs without males in spring. To elucidate the cause of sexual difference of such hibernating success, we compared super-cooling point (abbr. SCP) of the adults, because SCP is one of the good indicator of cold hardiness in insects.

SCP of the diapausing adult (grown under 16L-8D regime at 25°C) of *O. sauteri* was -24.7±2.3 days (N=30) in the female and -21.7 ±2.4 days (N=39) in the male. It indicates that the diapausing male has considerably low SCP, though the diapausing female has statistically lower SCP. A similar result was obtained in diapausing adults of *O. minutus*; SCP was -22.9 ±2.6 days (N=36) in the female and -22.3±2.7 days (N=35) in the male.

Since the lowest winter temperature seldom falls below -15°C in Sapporo, SCP of the diapausing males of both species seems to be enough to tolerate freeze. In conclusion, there are no biological differences in SCP between the sexes in both species. Factor(s) other than SCP may affect the sexual difference of winter survival.

20-146

DIFFERENTIAL NUTRIENT ALLOCATION TO REPRODUCTION AND SOMATIC MAINTENANCE BETWEEN TWO SPECIES OF *Orius* (HETEROPTERA: ANTHOCORIDAE)**Yoshitaka Nakashima and Yoshimi Hirose****Institute of Biological control, Faculty of Agriculture, Kyushu University, Fukuoka, Japan**

A laboratory experiment was conducted to determine the effects of egg production on the longevity of females of *Orius sauteri* and *O. tantillus*, predators of *Thrips palmi*. We compared their longevity between unmated and mated females because oviposition of both *Orius* species occurs only among their mated females. The results of the experiment showed that in *O. sauteri*, the longevity of mated females was half as much as that of unmated females, but that there was no difference in the longevity between mated and unmated females of *O. tantillus*. Daily fecundity of *O. sauteri* was twice as much as that of *O. tantillus*. It follows that egg production of *O. sauteri* results in a shortened longevity, whereas egg production of *O. tantillus* does not, and that the allocation of nutrients to reproduction and somatic maintenance differs between these two species. It is concluded that *O. sauteri* invested nutrients more heavily in its reproduction than *O. tantillus*.

20-148

HETEROPTEROUS PREDATORS OF THE PEAR PSYLLA (*CACOPSYLLA PYRI*) IN EASTERN SICILY**C. Rapisarda¹, S. Beninato², G. Campo²****1 Istituto di Entomologia agraria, Università degli Studi, Catania, Italy - 2 Osservatorio per le Malattie delle Piante, Acireale, Italy**

The Authors deal with the first results of a study which has been recently started on the arthropod fauna limiting the pear psylla (*Cacopsylla pyri*) populations, in a wide fruit area on the western slopes of Mount Etna. In particular, data are reported here on the activity of predacious Heteroptera, deriving from regular collections made by the method indicated as "battage" or "frappage", in various orchards where different pest management schemes were adopted. Emphasis has been given to the general study of the involved biocenosis, which showed the constant and predominant occurrence of *Anthocoris nemoralis*, compared to the other representatives of both the families Anthocoridae and Miridae. Comments are also given on the catches of all the species, in relation to the seasons, to the population trend of the pear psylla and the phytiatric management of the orchards.

20-147

FEEDING ACTIVITY, DEVELOPMENT AND REPRODUCTION OF *Orius majusculus* REUTER EXCLUSIVELY FED WITH *Thrips tabaci* LINDEMAN**C. Sengonca and M. Schade****Institute of Phytopathology, University of Bonn, FRG**

The polyphagous predatory bug *Orius majusculus* Reuter (Het., Anthocoridae) is a commercial product primarily sold for greenhouse use. Laboratory studies were conducted to determine its suitability for use as a predator against the important pest *Thrips tabaci* Lindeman (Thys., Thripidae) on field grown leek, onion and cabbage. Feeding activity, development and reproduction were studied in specially prepared bioassay chambers, that provided hiding places for the thrip larvae (L₂). All experiments were carried out with a fluctuating temperature of 24±1 °C and 17±1 °C at 16 h of artificial illumination and 8 h of darkness.

Female L₅-larvae showed the highest predatory activity with an average of 4.7 thrips larvae consumed per day. Male L₅-larvae only consumed 4.2 thrips larvae per day. Adult feeding activity was slightly lower. More than 400 thrips were consumed during the life cycle of a female bug. Complete female development took an average 51.6 days and the males 45.4 days. Almost 70 % of the predatory bugs developed fully on *T. tabaci*. The females of the first generation which had been fed exclusively on *T. tabaci*, laid 92.6 eggs during their oviposition period. The females of the F₁-generation, which had been exclusively fed with thrips, also achieved a significantly higher egg production level. They laid (?) An average of 131.0 eggs during the same oviposition period.

20-149

THE USE OF SPECIES *PODISUS MACULIVENTRIS* (SAY) (HETEROPTERA : PENTATOMIDAE) FOR BIOLOGICAL CONTROL OF COLORADO BEETLE IN THE POTATO CROPS FROM ROMANIA**T. MANOLE, M. IAMANDEI¹****Department of Entomology, Research Institute for Plant Protection, Bucharest, Romania-1Department of Entomology, Research Institute for Plant Protection, Bucharest, Romania**

In the present paper are included for the first time in Romania data concerning the use of species *Podisus maculiventris* (Say) in the biological control of larval density of Colorado beetle population in the potato crops including the four important agricultural regions from Romania.

The experimental dispositive was composed by 4 variant which are corresponding at the different levels of spreading. The results obtained are presented in the tables and shows a good efficiency in the control of the pest in comparison with the chemicals standard.

20-150

POSSIBILITIES OF BIOLOGICAL CONTROL OF NOCTUID EGGS BY THE MIRID *CYRTOPELTIS TENUIS* REUTER (HETEROP.: MIRIDAE).

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Periodical observations about the population dynamic of *Cyrtopeltis tenuis* were carried out on out-door tomato crops in Tenerife Island. The results showed a very good control of the noctuid *Autographa gamma* (Lep.: Noctuidae) eggs by the mirid and the correlation between the vertical and spatial distribution of both factors, mirids and eggs.

20-152

PHEROMONE-INDUCED MOVEMENT OF *Podisus maculiventris* (HETEROPTERA: PENTATOMIDAE) NYMPHS

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Field experiments were conducted at USDA (Beltsville, MD, USA) during the summer of 1995. The pheromone-induced movement of nymphs of the generalist predator spined soldier bug (*Podisus maculiventris* (Say) (Heteroptera: Pentatomidae), was determined by using pheromone releasers. The experimental field was planted with green beans (*Phaseolus vulgaris*), and divided in seven plots of 13 rows each. When the plants were three weeks old, plots were already naturally infested with Mexican bean beetles (*Epilachna varivestis* (Moulsant) (Coleoptera: Coccinellidae)), a common prey of *P. maculiventris*. At that time, third and fourth instars of the Spined soldier bug were released in the seventh row, and three pheromone releasers were placed outside the thirteenth row of each plot. The movement of the nymphs in either direction (i.e. towards the pheromone releasers or in the opposite direction) was determined three times in the lapse of one week. Results did not show nymphal movement 24 hours after release. However, the observations conducted in the fourth and seventh day after release showed a significant movement of the pentatomids in the direction of the pheromone releasers.

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KAIROMONAL ACTIVITY OF AGGREGATION PHEROMONE IN THE SYSTEM *Podisus maculiventris* (SAY)- *Telenomus* spp. (HETEROPTERA: PENTATOMIDAE; HYMENOPTERA: SCELIONIDAE)

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The kairomonal activity of the aggregation pheromone of *Podisus maculiventris* (Say), a predacious stink bug, has been tested in the field with two egg parasitoids, the Scelionids *Telenomus calvus* Johnson a specialist phoretic species, and *Telenomus podisi* Ashmead a generalist non-phoretic species.

Egg masses of *P. maculiventris*, obtained from a laboratory culture, were placed in traps (plastic cylinders with two funnel openings) baited with synthetic pheromone and exposed in the field. The egg masses of a phytophagous Pentatomid, *Euschistus obscurus* (Palisot), were also placed in the same traps to test the possible effects of the pheromone on acceptance behavior of both parasitoids. Traps of the same type with egg masses and without pheromone were used as controls. The traps were checked twice a week, rebaited and reloaded with freshly laid egg masses for about three months. The *P. maculiventris* population was monitored using the same traps from early spring.

Parasitized vs. unparasitized egg-masses were checked in the laboratory to assess the location and exploitation efficiency of the two *Telenomus* species. *T. podisi* did not respond to the pheromone whereas *T. calvus* was attracted by it and was capable of successfully attacking the host eggs without the phoretic phase, i.e. with no contact with the adult host.

The possibility of using the pheromone to manipulate the predator, when introduced in new environments, is discussed.

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PREY DEFENSES AFFECT THE PREY PREFERENCE OF THE PREDATORY BUG, *Eocanthecona furcellata* (WOLFF) (HETEROPTERA: PENTATOMIDAE: ASOPINAE)

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An asopine bug, *Eocanthecona furcellata*, is an effective predator of many insect pests in the Orient. The prey preference of *E. furcellata* was investigated with 71 insect species as prey, and it was found to be affected by some characteristics of the prey. *E. furcellata* tended to prefer the species that wandered extensively, had weak defences, a soft skin, no smell, no poisonous substance(s), and no projections, as compared to species stopped moving rapidly, had strong defenses, a hard skin, projections, a noxious odor, and/or a poison(s). From scores for these defensive characteristics of the prey, a simple discriminative index of the suitability for the predator in a closed environment is proposed for use in attempts at biological control. This study also provides some new host records for *E. furcellata* in Japan.

20-154

ROLE OF THE CHEMICAL CUES IN THE LARVAE OF *SPODOPTERA LITURA* IN PREY-LOCATION BEHAVIOR OF THE PREDATORY STINK BUG, *EOCANTHECONA FURCELLATA*.

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Behavioral responses of a predatory stink bug, *Eocanthecona furcellata* (Wolff) (Heteroptera: Pentatomidae) towards *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) larvae were observed in olfactometers. In dual-tube olfactometer, adult bugs were observed to approach and stretch the proboscis toward the air containing odors from intact *S. litura* larvae. The solvent extracts of larvae elicited similar behaviors. In a linear track olfactometer, nymph bugs were observed to be attracted to a fraction from silica gel column chromatography, but proboscis-protruding behavior was not observed toward this fraction. The proboscis-protruding behavior was, however, observed toward the other fraction by simple behavioral assay using a Pasteur pipette. These findings therefore indicated the presence of at least two different chemical cues in prey-location.

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APHELINUS SPECIES (HYMENOPTERA: APHELINIDAE), PARASITOIDS OF APHIDS (HOMOPTERA: APHIDIDAE) IN EUROPE

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The species of genus *Aphelinus* Dalman are known almost exclusively as primary endoparasitoids of aphids and are of importance in control of these pests.

Aphelinus species from European part of Palaearctic are revised, including the new materials. Twenty two species are recorded. Five species are recorded for the first time. Three of these: *Aphelinus brunneus* Jasnosh, *A. campestris* Jasn., *A. lucidus* Jasn., were earlier described from other parts of Palaearctic and two species - *A. fuscus* Chervonenko, *A. coracinus* Chervon. are described as new.

Notes on taxonomy, distribution, host range are discussed.

20-155

DEVELOPMENT OF *MACROLOPHUS CALIGINOSUS* AND *DICYPHUS ERRANS* REARED ON DIFFERENT DIETS (RHYNCHOTA MIRIDAE)

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The presence of several species of Miridae Dicyphinae has been checked in IPM vegetable crops in South Europe during the last years. These species, considered polyphagous predators and characterized by a zoophytophagous behaviour, were able to control whitefly infestations. In northwestern Italy the most abundant species were *Macrolophus caliginosus* Wagner and *Dicyphus errans* (Wolff).

In order to study the zoophytophagous behaviour and to ascertain the survival rate and the postembryonic developmental period of these two species, they were reared on three different diets: animal (eggs of *Ephestia kuehniella* Zeller), vegetal (French bean pods), mixed (eggs + pods), and at two temperatures: T1 (18-22°C), T2 (22-26°C). For each diet and each temperature, 15 specimens of the two mirids were reared in glass tubes and were daily checked.

M. caliginosus was able to complete its development on all diets, nevertheless it showed the highest rate of emergences on the mixed diet at both temperatures. On the contrary, *D. errans* was unable to survive on the vegetal diet, whereas it completed the egg to adult development when reared on the other two diets; the higher rate of emergences was observed on the mixed diet at both temperatures.

The postembryonic developmental period was influenced by diet and temperature in both species. Nymphs of *M. caliginosus* reared on the vegetal diet took a significantly longer time from hatching to emergence (50±6.66 days at T1 and 29.9±4.82 days at T2) than nymphs reared on the other two diets (from 31±2.94 days on the animal diet at T1 to 22.8±1.85 days on the mixed diet at T2). Nymphs of *D. errans* reared on animal and mixed diets became adults in significantly different times: longer on the animal diet (32±2.4 at T1 and 17.5±2.66 at T2) than on the mixed diet (28.5±2.32 at T1 and 15.5±1.36 at T2).

The mixed diet appeared the most suitable nourishment for rearing both species.

20-157

FAUNA OF APHELINIDS (HYMENOPTERA: APHELINIDAE) IN THE MOUNTAINOUS FRUIT ORCHARDS IN REPUBLIC OF GEORGIA

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Fruit orchards are on the south slopes of Caucasus. The principal are apple, pear and plum cultures.

The distribution of aphelinids is connected with their main hosts: coccid insects (Homoptera: Coccinea) and aphids (Homoptera: Aphidoidea). On 18 species of armored scales (Diaspididae) 13 species of aphelinids are registered, predominantly from the genera: *Aphytis* (5), *Encarsia* (2) and *Coccobius* (1). They are of importance in the control of *Diaspidiotus perniciosus* (Comstock), *Parlatoria oleae* (Colvée), *Epidiaspis leperii* (Signoret) and other pests.

For the control of Coccidae family pests (4) are more important: *Coccophagus palaeolecanii* Jasnosh and *C. lycimnia* (Walker).

More than 20 species of aphids and only 4 species of aphelinids, there parasitoids, are known in fruit orchards. More common are *Aphelinus mali* (Haldeman) and *A. chaonia* (Walker).

The fauna of aphelinids forms predominantly by European species and only some Asiatic species are here distributed.

The list of the Aphelinidae species and their hosts is presented.

20-158

PARASITIDS (HYMENOPTERA: CHALCIDOIDEA) AS BIOLOGICAL CONTROL AGENTS FOR THE WHITEFLY ALEUROCYBOTUS INDICUS DAVID & SUBRAMANIAM (HOMOPTERA: ALEYRODIDAE) ON RICE IN CHINA

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The whitefly of rice was first found in China in 1960, when it was identified as Bemisia sp. It is obvious that specimens of the whitefly of rice examined from Fujian Province of China are Aleurocybotus indicus David & Subramaniam. The whitefly of rice has recently become an important pest and is spreading to many other rice-growing areas in China.

Three parasitoids on the whitefly of rice have been found, in which Encarsia japonica Viggiani and Encarsia transvena (Timberlake) are dominant parasitoids with high percentage of population, and another species of Eulophidae is with very low population. Parasitism by the two species of Encarsia is generally in 6.68-33.04%, and 13.38% on an average. Parasitoid population reaches its peak in mid July and from late August to early September. Encarsia spp. are expected to be used as biocontrol agents for the whitefly of rice.

20-160

REPRODUCTIVE BIOLOGY AND MORPHOLOGY OF AN APHELINID PARASITOID ATTACKING THE SILVERLEAF WHITEFLY.

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The reproductive biology and the morphology of the reproductive system of Eretmocerus sp. nr. californicus that attacks silverleaf whitefly, Bemisia argentifolii Bellows & Perring are described. Ova morphology of Eretmocerus and Encarsia species is compared.

Demographic parameters such as fecundity, fertility, longevity, reproductive rates, and intrinsic growth rates are described for Eretmocerus sp. nr. californicus. Aspects of parasitoid oviposition behavior for E. sp. nr. californicus was analyzed on five host plant species. Adult wasps reared on sweet potato showed major differences in overall reproductive output of parasitoids exposed to different host plant species with differing leaf morphology. The most significant behavioral differences were the willingness of adult parasites to remain and search for whitefly hosts and the durations of host assessment and host feeding were also strikingly different among the host plant species.

The effects of the natal host plant on parasitoid searching and selection behaviors and percentage oviposition were tested and analyzed. Wasps raised on melon rather than sweet potato showed no significant differences in their performance on that host plant or the other plants species tested.

20-159

PARASITIDS (HYMENOPTERA, CHALCIDOIDEA) OF WHITEFLIES (HOMOPTERA, ALEYRODOIDEA) IN TURKMENISTAN
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At present in Turkmenistan are revealed 14 species of whiteflies. There are by our investigations 10 reliable parasitoid species of aleyrodids in Turkmenistan. They are belonging to 3 families of chalcidoid wasps: Aphelinidae (7), Eulophidae (2) and Encyrtidae (1 species). Hosts of these parasitoids are 8 whiteflies species. 5 species from them were not known before in Turkmenian fauna, including 4 species what were not known in Central Asia too.

Parasitoid's lists of main pests include: 6 species for Bemisia tabaci Genn., 5 species for Trialeurodes vaporariorum Westw. There are mainly species of genera Encarsia and Eretmocerus in these complexes. In conditions of Turkmenistan preferable hosts are: by Eretmocerus nikolskajae Myarts.- Bulgarialeurodes cotesi Mask., by Eretmocerus mundus Merc.- Bemisia tabaci, by Encarsia inaron How.- Aleyrodes proletella L.

20-161

INTRA- AND INTERSPECIFIC HOST DISCRIMINATION IN COCCOBIUS FULVUS (COMPERE ET ANNECKE) (HYMENOPTERA: APHELINIDAE), A PARASITOID OF UNASPIS YANONENSIS

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Coccobius fulvus and Aphytis yanonensis were introduced parasitoids from China to Japan for the biological control of the arrowhead scale, Unaspis yanonensis. To determine ability of intra- and interspecific host discrimination of C. fulvus, an intrinsically inferior species, oviposition behavior of the females toward hosts containing different developmental stages of conspecific and A. yanonensis were observed. They rejected hosts containing eggs, young larvae, old larvae and pupae of conspecific at 66.7, 20.0, 72.7 and 100%, respectively. The rejection of hosts containing eggs of conspecific was occurred before probing but the parasitoid rejected the hosts containing larvae or pupae after probing. It was suggested that the females used external cues to discriminate hosts with parasitoid eggs but they used internal ones when the hosts contained parasitoid larvae and pupae. On the other hand, the females rejected hosts containing eggs, young larvae, old larvae and pupae of A. yanonensis at 0, 25.0, 100 and 100%, respectively. It seemed that the external cues could not be detected by C. fulvus when the hosts had been parasitized by A. yanonensis. After the rejection of the hosts parasitized by A. yanonensis, 29% of the females fed from the host. The rejection of the hosts and the feeding from them could be adaptive behavior because C. fulvus was defeated in the case of multiparasitism.

20-162

NATURAL ENEMIES OF APHIDS AND THEIR POTENTIAL POWER IN TÜRKİYE

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Aphids are one of the most destructive agricultural pests and can cause significant damages direct or indirect ways for plants. However, the family of Aphididae (Homoptera) has also several natural enemies. Many studies have been done about the natural enemies of aphids by various entomologists in Türkiye. In the present study, all the previous records concerning the predators, parasitoids and diseases of aphids were compiled and listed.

In Türkiye 162 predatory insects in Coleoptera, Diptera, Neuroptera and Heteroptera orders, 38 parasitoids in Aphidiidae, Aphelinidae, Encyrtidae, Pteromalidae families (Hymenoptera) and an entomopathogen fungus were determined. In addition to the mentioned natural enemies 10 hyperparasite species and 19 parasite of the predators were found out. In the remaining part of the article certain knowledge on the potentiality of these beneficial insects was discussed.

20-164

SUCCESSFUL BIOLOGICAL CONTROL OF *Parabemisia myricae* (KUWANA) BY *Eretmocerus debachi* ROSE ET ROSEN IN THE EAST MEDITERRANEAN CITRUS-REGION OF TURKEYÇ. Şengonca¹, N. Uygun², U. Kersting², M.R. Ulusoy²¹Inst. of Phytopathology, University of Bonn, FRG and ² Dept. of Plant Protection, University of Çukurova, Turkey

The White Fly, *Parabemisia myricae* (Kuwana) (Hom., Aleyrodidae), which had been imported in the mid eighties, is a serious pest of citrus because of direct feeding damage as well as for its role as vector of citrus chlorotic dwarf (CCD) a severe disease in the east Mediterranean region of Turkey. To control this pest biologically, the aphelinid *Eretmocerus debachi* Rose et Rosen (Hym., Aphelinidae) was imported and mass released in the entire citrus-growing area. Within only three years the whitefly population was brought down to almost zero level. Even to smallest increases in pest populations, the parasitoid reacted with increasing parasitization. The high efficiency of *E. debachi* was also proved in laboratory experiments. The developmental time ranges from 12-28 days and the longevity lasted 2.1-3.7 days. Thus, *E. debachi* completed 13-14 generations a year while *P. myricae* passes only 7-8. *E. debachi* reproduced thelytokously, parasitizing about 53 individuals of different *P. myricae*-instars. A high mortality of *P. myricae* occurred due to host-feeding. The total mortality (parasitization and host-feeding) reached about 97 % for *P. myricae*-second instar nymphs. The biological control of *P. myricae* by *E. debachi* was a complete success and is one of few outstanding examples in biological control of whitefly species.

20-163

APHID-ATTACKING APHELINID SPECIES IN JAPAN: TAXONOMY, BIOLOGY AND BEHAVIOR.

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Two species of *Aphelinus* have hitherto been recorded from Japan. In the present study 13 other species are added to the fauna. These species are outlined and some information on their biology, and oviposition and host-feeding behavior is given. Discussion on the generic taxonomy will also made.

20-165

NATURAL ENEMIES OF WHITEFLIES IN THE DOMINICAN REPUBLIC - THEIR PROSPECTS FOR BIOCONTROL IN TOMATO AND ORNAMENTAL CROPS

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The Dominican mainly processing-tomato production in the irrigated lowlands has been severely threaten since 1988/89 by excessively high and insecticide-resistant populations of *Bemisia tabaci* and since 1991 by the transmission of geminiviruses as the tomato yellow leaf curl virus (TYLCV-Is). In order to enhance biocontrol as a component of IPM of the *Bemisia tabaci* complex and also *Trialeurodes vaporariorum* (in vegetables incl. fresh market tomatoes in mountain valleys), a nationwide survey on existing whitefly species (≥ 18 spp.) and associated enemies was carried (1995-96). Their potential for biocontrol mainly during the crop-free period is discussed.

Coccinellids (incl. *Delphastus*, spp.), chrysopids and other predators were recorded, but only mind bugs (*Cyrtopeltis tenuis*, *C. modesta*) occur in relative high densities after the critical initial weeks. Although natural parasitism is relatively low in existing tomato-growing systems, a diversity of parasitic aphelinids incl. *Encarsia transvena*, *Encarsia* sp. (*parvella* group, uniparental?), *En. pergandiella*, *En. formosa*, *En. meritoria*. (= *?hispidia*), *En. nigricephala* and *Eretmocerus* sp. nr. *californicus* have been reared from weeds and crops. *A. fuscipennis* (Hym.: Platygasteridae) a parasitoid of *T. vaporariorum* shows high parasitism levels ($\leq 95\%$) in unsprayed plants in mountain valleys. Endemic species have been compared in bioassays and field cages to biotypes imported from MBCC, Mission, Texas, in order to start mass rearing and liberation of the most promising parasitoids. Entomopathogenic fungi (Hyphomycetes-Moniales) *Paecilomyces fumoso-roseus* and *Verticillium lecanii* (≤ 500 and 400-1500 m.a.s.l. respectively) occur associated to *T. vaporariorum*. Commercial mycoinsecticides as well as selected endemic and introduced strains of different fungi species (incl. of *Beauveria bassiana*, *Entomophthora virulans*) were compared on tomato and *Gerbera* sp. plants.

20-166

COMPARISON OF SEARCHING EFFICIENCIES OF FIVE PARASITOID SPECIES (APHELINIDAE AND PLATYGASTERIDAE) OF *BEMISIA TABACI*/ARGENTIFOLII (ALEYRODIDAE).

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The first step in the selection of natural enemies for biological control is an evaluation based on qualitative parameters: seasonal synchronization with host; internal synchronization with host; climatic adaptation; no negative effects such as hyperparasitization; easy to rear. The second step is to rank these species with respect to their efficiency to depress the host and when inoculative control is intended to maintain the host below the economic threshold level. Searching efficiency is one of the factors that is hypothesized to be important for the efficiency of natural enemies in biological control, specifically at the low host densities occurring around the economic threshold level. For five parasitoid species of *Bemisia tabaci*/argenteofolii: *Encarsia formosa* Gahan (Aphelinidae), *Amitus bennettii* Viggiani & Evans (Platygasteridae); *Eretmocerus* nr *californicus* (Aphelinidae); *Er. mundus* Mercet. and a uniparental *Er. sp.* from Texas, we determined the walking speeds and walking patterns on poinsettia (*Euphorbia pulcherrima*, cv. Goldfinger) leaf discs before and after encounter of a host larva and before and after encounter of honeydew of the host. Data were recorded using a sampling rate of 8 frames per second with an automated system that directly translates the video picture into digital information. When no host cues are used, we expect random turning and no area restricted search. In this case the probability of encountering a host depends very much on the walking speed and the innate turning rate of the parasitoid. When host cues are used we expect some kind of area restricted search in case of contact infochemicals and directed search in case of volatile infochemicals. We will present the data for these species on the walking speed and walking pattern (amount of turning, amount of looping, number of times crossing its own path) and rank the species based on these parameters. Whether the ranking of the parasitoids at the level of the individual behaviour is also reflected in their efficiency at the population level will be evaluated using the simulation model developed in our laboratory.

20-168

BIOLOGY AND BIOCONTROL POTENTIAL OF *PEDIOBIUS FURVUS* (GAH.) FOR THE MANAGEMENT OF CEREAL STEM BORERS IN AFRICA.

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Pediobius furvus (Gah.) (Hymenoptera: Eulophidae) is a gregarious endoparasitoid of graminaceous stem borers in Africa. Investigations on the nature of trophic interactions between the plant, host insect and the parasitoid showed that no one stimulant alone performed the service of guiding *P. furvus* females to their proper host habitat, and the host location is achieved by a complex of array of stimuli working in harmony. Studies in the laboratory showed that low temperature resulted in longer developmental period, less progeny production and preponderance of males. Host and parasitoid age independent of size, were found to be one of the most important factors influencing the acceptability and suitability of both *C. partellus* and *B. fusca* pupae. Sucrose and/or honey concentrations as food for the adult parasitoid has played an important role in prolonging the longevity of the adult parasitoid but does not affect its fecundity. Studies on oviposition and through dissections reveal that the parasitoid is pro-ovigenic. Superparasitism in this parasitoid is accompanied by a reduction in size of the adult and preponderance of males which reduce the efficacy of the parasitoid. The rate of multiplication of *P. furvus* in one generation was found to be 335 and the mean length of the parasitoid generation recorded was 21 days. The species has a finite rate of increase as 1.31 per day at 25 ± 10 °C under 65 ± 1.5% RH.

20-167

PARASITIDS REARED FROM APHIDS ATTACKING CITRUS AND THEIR FREQUENCY IN GREECE

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A study concerning the knowledge on parasitoids, which attack aphids infesting various citrus, was undertaken since the spring of 1995 over the most important citrus growing areas of Greece. The samples taken were kept in suitable conditions to allow emergence of parasitoids from the aphid mummies, and then specimens were identified using appropriate keys.

The study revealed the presence of several parasitoid species belonging to genus *Aphidius*, *Trioxys*, *Lysiphlebus* and *Diaeretiella* (Hymenoptera, Aphididae). The parasitoids found emerged from various aphid species such as *Aphis spiraeicola* Patch, *Aphis gossypii* Glover, *Toxoptera aurantii* (Boyer de Fonscolombe) and *Myzus persicae* (Sulzer). *A. gossypii* was found to be parasitized by the most parasitoid species recorded whereas *Aphidius colemani* Viereck parasitized all aphid species which developed colonies on various citrus.

20-169

Distribution of Parasitism by *Lydella thompsoni* (Hert.) (Diptera: Tachinidae) in Maize Attacked by Stalk Corn Borer, *Sesamia nonagrioides* Lef. (Lepidoptera: Noctuidae)

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Abstract

Lydella thompsoni (Herting) (Diptera: Tachinidae) is a parasitoid of the stalk corn borer, *Sesamia nonagrioides* Lef. (Lepidoptera: Noctuidae). Over a 2-yr period, we collected stalk corn borer larvae from maize fields in Sorraia Valley (South of Portugal) to determine how host density and other factors influence the distribution of parasitism. The proportion of parasitized larvae was density dependent at the single-stalk and fields scales. Parasitism was always higher only in the 2nd generation of the trivoltine population. Proportion of parasitized larvae was correlated with the frequency of infested stalks. These relationships suggest that searching and oviposition (i.e. foraging) by the parasitoids may be among the maize stalks infested. The apparent efficiency of *L. thompsoni* in the 2nd generation of *S. nonagrioides* is discussed.

Key Words- *Lydella thompsoni*, *Sesamia nonagrioides*, biological control.

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ECOLOGICAL AND GENETIC INTERACTION BETWEEN AN INTRODUCED AND AN INDIGENOUS TORYMID PARASITOID IN BIOLOGICAL CONTROL OF THE CHESTNUT GALL WASP

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The chestnut gall wasp is assumed to have been introduced from China around 1940. *Torymus sinensis* was introduced from China into Japan and released at Tsukuba in 1982 for controlling this pest. After establishment, the parasitoid spreaded gradually in the Kanto Plains and caused a considerable reduction of the density of galls of the wasp. Before the introduction of *T. sinensis*, a closely related species, *T. beneficus*, was known to be an indigenous parasitoid of the wasp. Galls of the wasp were collected at several sites of the Kanto Plains and emerged adults of torymid parasitoids from the galls were checked for several years. As a result, it is likely that *T. beneficus* is being excluded by *T. sinensis* in this area. Isozyme discrimination was carried out to confirm possibilities for occurrence of interspecific crossing in the field between the two torymid species. The results suggest a low rate of interspecific crossing although hybrid individuals were produced in crossing experiments.

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CLASSICAL AND FORTUITOUS BIOLOGICAL CONTROL WITH ENTOMOPHAGOUS INSECTS IN ITALY

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From 1974 to 1994, 82 phytophagous insect species, not previously recorded in the Italian fauna, have been scored. Most of them are nonindigenous pests against which some natural enemies have been introduced.

Six exotic entomophagous species have been used in planned programs while 13 have been found to exert some fortuitous control. Against 24 pests, including indigenous and long established exotic ones, 33 species of entomophages have been recorded. Of these, 22 were utilized using a classical approach and 11 had acted in a fortuitous way.

On average, over 20 years, host/entomophage fortuitous associations have occurred with a rate of 1.1 per year, while in planned programs the rate was 1.4. In a previous review (1974), reporting only the classical approach, a rate of about 0.7 was scored.

Efficacy was defined, when data were available, according to the usual parameters, i.e. complete, substantial and partial control. When establishment of an introduced species failed, a qualitative analysis of causes was attempted but definite results were not obtained because of lack of information and lack of follow-up studies. Therefore the adoption of rigorous protocol procedures to improve the success rate or explain failed introductions is recommended.

20-171

BIOLOGICAL CONTROL EXPLORATION AND RESEARCH BY THE OFFICE OF INTERNATIONAL RESEARCH PROGRAMS, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

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1 Office of International Research Programs, Beltsville, MD USA - 2 European Biological Control Laboratory, Montpellier, France - 3 South American Biological Control Laboratory, Hurlingham, Argentina - 4 Australian Biological Control Laboratory, Brisbane, Australia - 5 Sino-American Biological Control Laboratory, Beijing, China. Permanent laboratories and staff are maintained in France, China, Argentina, and Australia as bases for exploration and research on natural enemies of major insect pests and weeds immigrant to the United States. In addition to exploration for new natural enemies, the 4 laboratories ship natural enemies to U.S. cooperators via quarantine labs in the U.S., study basic biologies, emphasizing host range testing and any other information needed for implementation of the biocontrol agents in the U.S. Cooperative linkages with host country and other national labs and researchers, including training of students, are emphasized. OIRP Headquarters also funds short-term explorations by U.S. researchers.

20-173

SCYMNUS COCCIVORA AIYAR (COLEOPTERA:COCCINELLIDAE) AN INDIGENOUS PREDATOR OF GRAPE MEALYBUG

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Mealybug, *Maconellicoccus hirsutus* Green is the most devastating species in grape ecosystem. During surveys the coccinellid, *Scymnus coccivora* Aiyar was found feeding on eggs and crawlers of *M. hirsutus* in the field.

The effect of temperature on the developmental duration of *S. coccivora* was quantified by deriving a regression equation for each developmental stage as well as the total life cycle. Daily/overall consumption of prey by predator was established. Preference of the predator to *M. hirsutus* compared to other mealybugs was evident. Based on these biological aspects the mass culturing of *S. coccivora* was standardised.

Further, to conserve the naturally existing population several pesticides (five insecticides, one acaricide and three fungicides) were evaluated against *S. coccivora* adults to determine safe pesticides along with waiting periods by calculating the median lethal time (LT) values in days following probit analysis. Dichlorvos 0.1% and tridemorph 0.05% were found to be safest insecticide and fungicide, respectively and can be integrated with predator release. Synthetic pyrethroids and triadimefon (a systemic fungicide) were highly toxic and foils the additional advantage of utilisation of bio-agent.

20-174

OVIPOSITION STRATEGIES OF LADYBIRDS (COLEOPTERA: COCCINELLIDAE) IN THE SPRING ALFALFA FIELD

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In the spring alfalfa field, the overwintered adults of *Coccinella septempunctata brucki* began their oviposition in the early spring when the aphid density was still low. *C. septempunctata brucki* laid their eggs mainly on undersides of dead leaves in the field. *Harmonia axyridis* began oviposition in the middle spring when the aphid density was rather high. *H. axyridis* preferred undersides of alive leaves of alfalfa for their oviposition sites.

In the laboratory condition, *H. axyridis* laid their eggs mainly on alfalfa. On the contrary, *C. septempunctata brucki* scarcely laid their eggs on alfalfa nor on the sites conditioned with their excretion.

The oviposition style of *C. septempunctata brucki* is seemed to escape the egg cannibalism by adults and larvae searching aphids on the alfalfa under the low aphid density. *H. axyridis* has less fear of egg cannibalism under the high aphid density and they lay their eggs on the alfalfa.

20-176

AUGMENTATIVE BIOLOGICAL CONTROL OF GRAPE MEALYBUG, *PSEUDOCOCCUS MARITIMUS* (EHRHORN) (HOMOPTERA: PSEUDOCOCCIDAE), IN PEAR

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Over the past several years the grape mealybug (GMB), *Pseudococcus maritimus* (Ehrhorn), has become an increasingly important pest of pear in the northwestern US. Reasons for the enhanced pest status include depletion of the natural enemy pool due to historically heavy pesticide use, increased resistance to organophosphate pesticides, and the cancellation of labels for pesticides previously used to control GMB.

The potential of two endemic biological control agents was examined using inclusion cages into which different densities of natural enemies were placed along with established populations of GMB. The experimental blocks were untreated with insecticides. The green lacewing, *Chrysoperla rufilabris* (Burmeister) significantly decreased GMB densities on infested pear branches. Another very common predator, the European earwig (*Forficula auricularia* L.) also significantly reduced GMB populations. Grape mealybug egg mass densities were significantly reduced by *C. rufilabris* or *F. auricularia* releases. Mass releases of lacewing eggs on GMB-infested trees resulted in decreased GMB numbers on the release trees and surrounding trees. There were few endemic predators or parasitoids present in GMB-infested pear blocks treated with insecticides or adjacent to pear or apple orchards under conventional spray programs.

20-175

THE FUNCTIONAL RESPONSES OF TWO *HYPERASPIS NOTATA* MULSANT (COLEOPTERA: COCCINELLIDAE) STRAINS TO THEIR PREY, THE CASSAVA MEALYBUG *PHENACOCCLUS MANIHOTI* MATILE-FERRERO (HOMOPTERA: PSEUDOCOCCIDAE)B. Stäubli Dreyer, J. Baumgärtner¹, P. Neuenschwander², S. Dorn³

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The number of prey individuals attacked by single larvae and adults of the coccinellid *Hyperaspis notata* Mulsant as affected by the density of its prey the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, was studied in the laboratory. The parameters of a demand-driven functional response model for two strains of *H. notata*, one originating from Southern Brazil and one from Colombia, were compared. In the case of larvae there were no differences between the Brazilian and the Colombian strain. Adult females of the Colombian strain, however, showed a greater searching rate but a lower prey demand rate than females of the Brazilian strain.

20-177

THE BIOLOGY OF THE MEALYBUG, *NIPAECCUS VIRIDIS* (NEWSTEAD), (HOMO.: PSEUDOCOCCIDAE) AND EVALUATION OF THREE LADYBIRDS SPECIES (COL.: COCCINELLIDAE) IN ITS BIOLOGICAL CONTROL IN SOUTHWEST, IRAN. Mossadegh, M.S. & A. Khodaman

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The mealybug, *Nipaeccus viridis* (Newstead) is one of the major pests of citrus spp. in Khuzestan province, southwest, Iran. In a two years survey (1991-93), the biology of the mealybug, was studied in the field and laboratory conditions. The biological aspects of *N. viridis* were studied on sprouted potato tubers at 20±1.30°C. and 26±1.30°C., 70±5%RH and under photoperiod LD 12:12. This mealybug completes 8 generations in a year, 7 during march-December and 1 during December-March at Ahwaz, conditions. Overlapping of successive generations took place and it over winters as egg, nymph and adult. It had two populations peaks in spring and autumn. The exotic predator *Cryptolaemus montrouzieri* Mul. and two other indigenous coccinellids *Hyperaspis polita* Weise and *Scymnus includens* Kirsch evaluated in biological control against *N. viridis* in the natural conditions. A number of young citrus spp. trees were infested with the mealybug crawlers in April 1992. To eliminate other biological agents, they were covered with white fabrics which were fixed on metal frames. Releasing of the coccinellids accomplished twice in April 1993. all the infested and control trees were sampled randomly in the end of December 1993. Analysis of data revealed the potential and capability of the three species of ladybirds in suppressing the mealybug population. However, *H. polita*, effectively acted better than other two species.

20-178

EFFECTS OF EXPERIENCE REGARDING PREY AND PLANT STRUCTURE ON PREDATION EFFICACY OF *COLEOMEGILLA MACULATA* LENGI TIMBERLAKE (COLEOPTERA: COCCINELLIDAE) ON *LEPTINOTARSA DECEMLINEATA* (SAY) (COLEOPTERA: CHRYSOMELIDAE)

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Predation efficacy of *Coleomegilla maculata* fourth instars on *Leptinotarsa decemlineata* young instars was investigated to look at the effects of experience in regard of the prey and the plant structure. Rearing conditions (artificial diet vs *L. decemlineata*; plant structure vs plastic container) did not affect the number of prey attacked over a 24h period. However in the first 3h, predators reared on artificial diet attacked significantly more prey than predators reared on *L. decemlineata*. This difference was not observed between naive predators and *L. decemlineata*-reared ones that had been put on artificial diet for 72h. Further investigations showed (1) that level of experience regarding the plant structure affected space and time partitioning of searching behavior, and (2) that prey-experimented predators needed more contacts with prey in order to attack, and rarely consumed the prey totally. Development of a food aversion towards *L. decemlineata* is likely to explain these results.

20-180

CRITICAL ISSUES IN REARING APHIDOPHAGOUS COCCINELLIDAE

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While in the field crops fostering native populations of aphidophagous ladybeetles is preferred, inundative releases are necessary for biocontrol in glasshouses; mass cultures are then a prerequisite. Three phenomena are critical in artificial rearing: (1) diapause, (2) prey specificity and (3) cannibalism.

(1) Continuous rearing can be achieved by the use of photoperiods preventing diapause, i.e. long daylength or increase in daylength in most species from cold temperate Europe, but short daylength in, e.g., *Coccinella septempunctata brucki* in central Honshu, Japan. In the species with strong tendency to univoltinism, this tendency can be gradually eliminated by selection, or multivoltine populations from other climatic region may be used when founding the culture. However, diapause can be used to our advantage for easier storage of viable adults; they can be produced by an appropriate sequence of feeding on aphids and alternative foods, based on honey or pollen.

(2) Successful rearing (high fecundity, high survival during larval development) on non-aphid food (eggs of Lepidoptera or Coleoptera, lyophilized larvae of honey bee males, plant pollen) can be achieved only in polyphagous species (e.g. *Coleomegilla maculata*, *Harmonia axyridis*). For oligophagous species (e.g. *Coccinella* spp.) certain aphids are less suitable or even toxic, usually due to allelochemicals from the host-plants of their aphid prey.

(3) Cannibalism can be limited by steady presence of surplus of prey, and providing much "walking" surface in the rearing cages.

(For more details see Hodek I. & Honek A., Ecology of Coccinellidae, 1996, Kluwer.)

20-179

RECORDS OF COCCINELLIDAE (COLEOPTERA) SPECIES PREYING ON COCONUT PESTS IN THE STATE OF ALAGOAS, NORTHEASTERN REGION OF BRAZIL: *OLLA V-NIGRUM* (MULSANT, 1866) AND *CYCLONEDA SANGUINEA SANGUINEA* (L., 1763) ON *CERATAPHIS* SP (HOMOPTERA, APHIDIDAE) AND *CHILOCORUS NIGRITA* (FABRICIUS, 1798) ON *ASPIDIOTUS DESTRUCTOR* (SIGNOTRET, 1869) (HOMOPTERA, DIASPIDIDAE)

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O. v-nigrum (Coccinellinae, Coccinellini) is well known as an aphid and psyllid predator. This is the first time that it was found preying on *Cerataphis* sp (Homaphidinae, Cerataphidini). All the life stages were collected in the field. Among the adults two color patterns were found for the species: the melanic form (more common) and the pale form. In *C. sanguinea sanguinea* (Coccinellini), primarily an aphid predator, all the life stages were detected in the field but in a lower density than in *O. v-nigrum*. *C. nigrata* (Chilocorinae, Chilocorini) an oriental Coccinellidae used as an introduced biocontrol agent of Diaspididae species in many regions of the world, was found preying on *A. destructor* a serious pest on young coconut palms. Its abundance and its presence on scale colonies on various plant species, could be an indicator of the establishment of this species. The improvement of its efficiency in the field could be reached by releasing adults from mass rearings. For *C. nigrata* simple techniques could be applied by using *Diaspis echinocacti* (Bouché, 1833) (Diaspidinae, Aspidiotini) growing on Cactaceae species - *Nopalea cochenillifera* (L.) Salm.-Dick and *Opuntia ficus-indica* (L.) Mill - as a food resource for the Coccinellidae. As *O. v-nigrum* and *C. sanguinea sanguinea* preying *Cerataphis* sp is a record, it is necessary to study possibilities of its rearing in laboratory conditions to be released in crop fields with infestation problems.

20-181

EFFICIENCY OF *Hippodamia convergens* GUERIN-MENEVILLE AS A PREDATOR OF *Thrips tabaci* LINDEMANN

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In order to develop specific biological control methods, suitable for target pest, sufficient knowledge about the biology and ecology of the beneficial insect is very important. Hence laboratory studies were conducted on the development, feeding activity and prey preference of *Hippodamia convergens* Guerin-Meneville (Col., Coccinellidae) as a potential predator of *Thrips tabaci* Lindeman (Thys., Thripidae). Since this predator is primarily provided for field releases against thrips on leek, experiments were carried out in specially prepared bioassay chambers designed for the prey to prevent possible predation. All experiments were conducted at 16 h of artificial illumination at a temperature of 24±1 °C and 8 h of darkness at 18±1 °C. In the control chambers *H. convergens* was exclusively fed aphids.

H. convergens developed fully on *T. tabaci*. Larval mortality was considerably higher than in the control. It reached an average of 48 % in the first two larval instars, whereas there was only 10 % mortality in the control. Development was clearly delayed taking 32.0 days on thrips, but only 23.8 days on aphids. Furthermore, constant feeding on thrips resulted in considerably smaller adults. The females laid pale-yellowish eggs, which developed normally. Feeding activity increased continuously during development and reached more than 200 thrips per day for L₄-larvae and more than 300 for the adults. There was a slight increase in the preference for the predation of aphids when compared to the predation of thrips.

20-182

SEASONAL DISTRIBUTION OF COCCINELLIDAE PREYING APHIDS IN AN INTENSIVELY CULTIVATED AGROECOSYSTEM (COLEOPTERA - RHYNCHOTA: APHIDOIDEA)

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The seasonal movements of Coccinellidae were investigated in the simplified biotic communities of a plain agroecosystem in northern Italy, with the aim of improving the natural control of aphids. For five years, collections by frapping and visual samples were systematically used to identify the preferred sites of overwintering and reproduction. Although fluctuations were recorded, three general trends were observed. 1) In springtime, adults regularly move from winter shelters to infested plants where they feed and reproduce. Wheat is the principal extensive crop colonised by *Adonia variegata* (Goeze), *Propylaea 14-punctata* (L.), and *Coccinella 7-punctata* L.; peach trees are often preferred by *Adalia 2-punctata* (L.) and some Scymnini; white poplar and blackthorn by *A.2-punctata*, Scymnini, *A.variegata*, and *Synharmonia conglobata* (L.); stinging nettles by *A.variegata*, Scymnini, *A.2-punctata*, and *C.7-punctata*. 2) In June and July, newly-emerged adults colonise the summer crops and start the second generation. Corn, sugar beat, sorghum, melon, watermelon, and other vegetables are preferred by *A.variegata*, *P.14-punctata*, *C.7-punctata*, and Scymnini; pear, apple, and peach trees mainly by *P.14-punctata*, *A.2-punctata*, and Scymnini. 3) At the end of summer, when extensive crops are harvested and many other plants dry out, adults leave the inhospitable canopies and move to hedges, grass-lands, and orchards, preparing for overwintering. Alfa-alfa appears to be the unique crop supporting large amounts of Coccinellidae during the year because it is regularly re-colonised after each cutting. In general, the polyvoltine species play a primary role in the natural control of aphids and the possibility of forecasting the colonisation times enhances the Integrated Pest Management in several crops. Biodiversity is essential for the permanence of predators in the agroecosystem, although wild plants are rarely significant reproduction sites, due to the limited surface and the generally low aphid infestations.

20-184

REARING METHOD FOR PREDATORY FLIES OF THE GENUS *COENOSIA* (MG.) (DIPTERA: MUSCIDAE) - NEW BENEFICIALS OF BIOLOGICAL CONTROL

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The development of a rearing method for the predatory flies *Coenosia atra*, *C. attenuata*, *C. humilis* and *C. strigipes* will provide a new group of beneficials to biological pest management in glasshouses.

Their prey range comprises for instance white flies, (Aleurodidae), sciarid flies (Sciaridae) and leaf-miners (Agromycidae). The predatory

Coenosia larvae feed on larvae of sciarid flies (Sciaridae: *Bradysia paupera* Tuomikoski) for which we have developed a specific rearing method. In contrast to all known methods larvae feed on a nutrient fungus which is cultivated in a wood-fibre-bark substrate.

Investigations have shown that sciarid flies lay their eggs on fungal mycelium and that the larvae prefer fungal hyphae of the genera *Fusarium*, *Botrytis* and *Alternaria*. *Coenosia* females lay their eggs on the surface of this rearing substrate. The predatory larvae of *C. strigipes* hatch after 5 days at 25°C and pupate after approx. 11 days.

After another 11 days imagines hatch. Commercial production of the flies will start in Germany in 1996.

20-183

FEEDING PREFERENCE, BETWEEN APHID AND COLLEMBOLAN PREY, OF AN ABUNDANT CARABID BEETLE, *PTEROSTICHUS CUPREUS*, ASSOCIATED WITH CEREAL FIELDS.

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In five different treatments the feeding preferences of *Pterostichus cupreus* adults (L), (Coleoptera, Carabidae) for the cereal aphid, *Metopolophium dirhodum*, (Walk.), or the Entomobryid collembolan *Heteromurus nitidus* (Templeton), was examined. *P. cupreus* exhibited higher consumption rates of aphids than of collembolans when offered both prey types simultaneously. Conversely, the consumption rate of collembolans was higher than of aphids when prey types were offered separately. Total consumption rates were not significantly lower when *P. cupreus* was offered both prey items simultaneously. In all treatments in which both prey items were presented simultaneously, the percentage of grid squares where *P. cupreus* consumed aphids first, was always significantly greater ($P < 0.05$). Beetles given a 5 day pre-experimental diet of aphids only, did not have a significantly different consumption rate of either prey type nor was there a significant change in percentage of squares where aphids were consumed first. Beetles given a 5 day pre-experimental diet of collembolans only, had a significantly lower consumption rate of aphids, ($P < 0.05$) and a lower percentage of grid squares where an aphid was consumed first ($P < 0.05$). It was concluded that aphids were consumed in preference to collembolans under the experimental conditions, and that prey selection can be affected by the diet previous to prey selection.

20-185

GREEN LACEWINGS (NEUROPTERA: CHRYSOPIDAE) IN DALMATIAN OLIVE ORCHARDS

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Neuroptera of Croatia, especially Neuroptera of Dalmatia, are only a few insufficiently investigated. Particularly, there are data about distribution and beneficial activity of Neuroptera from the agricultural point of view

In order to collect the first detailed information on the Neuroptera Chrysopidae, present in Dalmatian olive orchards, four experimental sites (Podgora, Duilovo, Kaštel Stari and islands Sit and Žut - Komati archipelago) were selected to be investigated during the vegetation periods in the years 1990 and 1991. In these orchards, chemical treatments were not applied and olive trees were attacked by *Prays oleae* Bem. and *Saissetia oleae* Oliv. Therefore those sites were rich with fauna from family Chrysopidae.

Our aim was to establish the list of our autochthonous Chrysopid species and investigate some morphological and biological characteristics.

Standardized and auxiliary collection methods of adults were used (Klopimethode - Steiner 1962, Mc Phail Traps for monitoring of *Dacus oleae* Gmel., Fluorescent Yellow Traps and Catching Lamp). We observed that Mc Phail traps, filled with hydrolysate protein or $(\text{NH}_4)_2\text{HPO}_4$, caught Chrysopid species which were not predaceous in the adult stage.

At the experimental site of Podgora six species were found, while in other sites seven species were identified. The most frequently species found was *Chrysoperla carnea* Steph. which was the only one that had been present throughout the year in Dalmatia. The less frequent species were *Mallada zelleri* Sch. and *Chrysopa pallens* Ramb.

The dynamics of flight and the share of various species were additionally investigated in orchards Duilovo and Kaštel Stari and will be presented.

20-186

THE ROLE OF SPIDERS AS NATURAL CONTROL AGENTS IN A COTTON AGROECOSYSTEM FROM ARGENTINA

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The relevance of spiders as natural enemies of insect pests have been evaluated during two years, in the framework of an Integrated Pest Management Program of Cotton Insects developing at the Experimental Agronomic Station of Presidencia Roque Sáenz Peña (Chaco, Argentina).

The records of biodiversity, abundance and distribution of spiders in sprayed and unsprayed fields were compared. Thomisidae, Araneidae, Philodromidae and Lycosidae were the dominant spider families in the crop. The present results clearly demonstrate that spraying of insecticides caused a serious drawback in spider populations.

Laboratory bioassays were carried out to observe spiders prey preferences for three Lepidoptera larval instars (*Alabama argillacea*, *Heliothis virescens* and *Spodoptera frugiperda*) and for a cotton bug, *Dysdercus chaquensis* (Heteroptera). The standard reference preys were *Musca domestica* or *Drosophila melanogaster* (Diptera). These assays showed that spiders preferred Lepidoptera larvae. Only Araneids fed upon *Dysdercus chaquensis*.

Consumption rates of *Heliothis virescens* larvae by *Misumenops pallens* (Thomisidae) were also determined. Males and females of *Misumenops pallens* consumed daily mean rations of 0.95 mg and 6.15 mg of *Heliothis virescens*, respectively.

20-188

COMPARATIVE CONSUMPTION LEVEL OF *CHRYSOPERLA EXTERNA* (HAGEN) AND *CERAEOCHRYSA PARAGUARUA* (NAVAS) (NEUROPTERA: CHRYSOPIDAE) FEEDING *HELIOTHIS VIRESCENS* (F.) (LEPIDOPTERA: NOCTUIDAE) EGGSM. Polak¹, A. Ruiz¹, G. Contreras¹, G. Videla¹, E. González Olazo²¹ Departamento de Entomología, Instituto Nacional de Tecnología Agropecuaria, Sáenz Peña, Chaco, Argentina² Fundación Miguel Lillo, Tucumán, Argentina

Samplings performed in the Domo Central Agrícola Chaqueño area of Argentina, using nets and light traps from 1992 to 1995, showed that *Chrysoperla externa* (Hagen 1856) and *Ceraeochrysa paraguaria* (Navás 1924) are the most abundant Chrysopidae present in cotton crops.

The objective of the present study was to comparatively measure the consumption of *Heliothis virescens* eggs performed by both species.

Tests were conducted under controlled conditions (25±3°C; 75±10% RH; 14:10 L:D). Experimental units were represented by plastic containers with three compartments of 2x2x1 cm each. Eggs of either species were individually placed in each compartment. Hatched larvae were fed *H. virescens* eggs *ad-libitum*. Consumption was registered by day and by stadium.

Each *C. paraguaria* larva consumed a total average of 628.9 (±44.9) eggs of the prey during the 13.5 (±0.7) days the larval stage lasted in average. *C. externa* larvae individually consumed a total average of 519.5 (±63.5) prey eggs during the 10.8 (±1.7) days the larval stage lasted in average. Both species significantly differed with respect to total consumption and duration of larval stage. Significant differences in consumption were also found among stadia within species and between each stadium of both species.

20-187

A NEW METHOD TO QUANTIFY THE PREDATION BY POLYPHAGOUS PREDATORS IN THE FIELD BY MARKING THEIR PREY WITH THE STABLE ISOTOPE ¹⁵N

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In various publications polyphagous predators are described to have an influence on the population level of agricultural pests like aphids, but the quantification of this impact in the field is difficult. Until now methods like electrophoresis, ELISA and enclosure-experiments have been used, yet all of them entail some problems with the interpretation of the data.

An alternative approach is marking the prey and following up the marker in the predators. Commonly used markers are radioactive isotopes and rare elements, but they can influence the behaviour of the prey. Furthermore radioactivity can cause contamination of the environment. The use of stable isotopes for the marking can help to avoid these problems: they are safe because they do not cause contamination and they do not alter the behaviour of the marked animals.

We propose a new method in a model system which consists in marking cereal aphids of the species *Sitobion avenae* (Homoptera: Aphididae) by increasing their natural level of ¹⁵N by feeding, and detecting later increased levels of ¹⁵N in polyphagous predators that ingested marked aphids by mass spectrometry. As predators we used *Platynus dorsalis* (Coleoptera: Carabidae) and *Erigone atra* (Araneae: Linyphiidae). The level of the tracer detected in these predators did not decline for at least 10 days after a marked prey was ingested, an important difference to other methods like ELISA where the prey can be detected only for shorter periods. First results of these and additional experiments are presented and the suitability of this method for the quantification of predation in the field is discussed.

20-189

'FORFICULA DECIPIENS' GENE', A NEW MULTIPREY PREDATOR FOR BIOLOGICAL CONTROL: BIOLOGICAL AND ETHOLOGICAL ASPECTS

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Forficula decipiens Genè is a species of Dermaptera diffused in the Mediterranean area. The trophic habit of the young larvae is prevalently insectivorous but pollen and fungal spores may be feeded. Contrary to *Forficula auricularia* L., closely related species *F. decipiens* never has been recognized as a dangerous pest of cultivated plant. In this work is demonstrated that the sub-social behaviour of the *F. decipiens* larvae gives the opportunity to employ the predator in familiar groups located in wooden nests attached at the bottom of the trunk. Preliminary trials demonstrate the ability of the earwigs (second and third larval stage) to prey aphids (young and adult stage), lepidoptera eggs, red spidermites, larvae of tripids and whitefly larvae.

In laboratory, a large colony of *F. decipiens* was reared in order to obtain a mass production of the predator. In the field it was checked the ability of this earwig to control: aphids infestation on peach plant (3 years of observations); *Lobesia botrana* Schiffmüller attacks on vineyards (3 years of observations); red spidermite infestation on the strawberry (1 year of observations). CONCLUSIONS: The study carried out candidates the earwig *Forficula decipiens* to be a new multiprey predator of many important pest.

20-190

CLASSICAL BIOLOGICAL CONTROL OF CASSAVA GREEN MITE *MONONYCHELLUS TANAJOA* (BONDAR) IN AFRICA AND SOUTH AMERICA BY PHYTOSEIID MITES (ACARI: TETRANYCHIDAE, PHYTOSEIIDAE)

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The cassava green mite feeds on leaves and growing tips of cassava *Manihot esculenta* Crantz, an important tropical root crop. The mite is native to the neotropics but became established in Africa in the early 1970s, where it causes extensive damage. It is also a serious pest in the dry region of northeast Brazil, where there are few species of predators.

CIAT, EMBRAPA and IITA have been collaborating to find suitable natural enemies in South America to release in Africa and northeast Brazil. This involves matching the climate of target regions to identify regions to explore and evaluating candidate predators in the laboratory for prey specificity, adaptation to dry climate, and efficacy. Ecological data collected during explorations is also used to describe the predators' ecological niches. Over 25 new species of predaceous phytoseiids have been described, and taxonomic keys are being developed.

Three species of phytoseiids have been established in Africa. Of these, *Typhlodromalus aripo* de León *sensu lato* and *T. manihoti* (Moraes) are spreading rapidly and are reducing cassava green mite populations significantly. Two species released in northeast Brazil have been recovered in small numbers.

20-192

VEGETATION AND CARABID FAUNA AFFECTED BY CONVENTIONAL AND BIOLOGICAL CULTIVATION

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In southwestern Finland, 6755:311 (Uniform Grid System, 27E), the cover of cultivated and noncultivated plants was measured in a conventional and biological crop rotation experiment in 1992-1995. The Carabid beetles were sampled during the season using pitfall traps in 1991-1995.

The crops grown were winter rye, pea and oats as mixed crop, barley as a cover crop for first year gramineous hay, first year grass and second year grass. The size of every plot was about 0.6 hectare. Conventionally cultivated plots were fertilized by inorganic fertilizers and sprayed with herbicide. The biologically cultivated plots were organically fertilized and not sprayed at all.

The total cover and the cover of cultivated plants was higher in the conventionally cultivated areas.

The weed cover and the number of weed species was higher in the biologically cultivated areas. Altogether in the plots there were the cultivated plants and 47 weeds.

The total Carabid catch was 26 species and 9682 specimens. 54 % of the specimens were collected from biologically cultivated. The most abundant species during 1991-1995, representing 67 % of the whole catch, were *Pterostichus cupreus* L., *Pterostichus melanarius* Ill., *Bembidion guttula* F., *Bembidion gilvipes* Sturm and *Amara* sp. The composition of species did not differ between cultivation strategies. *Bembidion*-species were dominant in summer 1991 and *Pterostichus*-species in summer 1993 and 1995.

20-191

SPIDER POPULATIONS IN A TYPICAL FIELD SITE IN CENTRAL GERMANY AND SPECIAL INFLUENCES OF VARIOUS PLANT PROTECTION INTENSITIES DURING A CROP ROTATION SEQUENCE (1991 - 1995)

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From 1991 to 1995 field investigations were carried out on a site (Obhausen, Querfurt county) in the central German dry region. This agricultural area stands out for its highly productive black earth soils (loess over loam) with relatively poor annual precipitation as the limiting factor. The crop rotation sequence winter barley (break in spring 1992 because of drought), maize, winter wheat, winter wheat, winter rye was studied on a 43 ha field. For this purpose 6 plots sized 72 x 200 m each were established. On two control plots no plant protection measures were applied. Another two were intensively treated with herbicides, fungicides and insecticides. On the remaining two plots targeted control measures were carried out with regard to threshold values. The epigeous spiders were sampled by using 9 pitfall traps per plot.

Altogether (1991 to 1995) the 68 805 spiders belonged to 104 species of 17 families. In maize herbicidal treatments in May and June did not directly influence the activity density of the spiders. In winter wheat the control variants revealed the highest activities (average 4,5 spiders per day and trap). There was no statistical difference to the integrated pest management variant (\bar{x} 4,1 spiders per day and trap). Especially the field species *Oedothorax apicatus*, *Erigone atra*, *Erigone dentipalpis*, *Meioneta rurestris*, *Porrhomma microphthalmum*, *Pardosa agrestis* and *Pardosa pratvaga* which are well adapted to open habitats reached high dominance levels on the control plots and on specifically treated field sections. Their long retention time in the field, the early presence in spring and their predatory performance makes spiders interesting objects in the development of beneficial thresholds. Summarizing we can conclude that purposeful use of plant protection agents with consideration of damage thresholds tend to leave a major number of spiders in their natural habitat.

20-193

BIOLOGICAL CONTROL AGENTS OF MUSK THISTLE, *CARDUUS NUTANS* (COMPLEX) IN THE UNITED STATES.

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Classical biological control of weeds uses introduced insects or pathogens to reduce the abundance of a weed to below its economic threshold. Musk thistle, *Carduus nutans* L., complex (Asteraceae), is a spiny, noxious herbaceous plant that chokes pastures, rangelands, and roadside areas. In the United States this weed is successfully controlled in many middle Atlantic and Midwestern states by the release of host specific insects. These insects are collected in Europe by personnel of the USDA/ARS Biological Control Laboratory - Europe. Two weevils, *Rhinocyllus conicus* (Froelich) and *Trichosiromus horridus* (Panzer), are responsible for most of the reduction in musk thistle density. Two flies, the syrphid, *Cheilosia corydon* (Fallén), and the tephrid, *Urophora solstitialis* (L.) are recent introductions for use against musk thistle in other areas of the country but are not yet well established. A strain of the rust fungus, *Puccinia carduorum* Jacky, also was tested and is ready for distribution in the Plains area.

20-194

BIOECOLOGICAL ASPECTS OF *LAMPROSOMA AZUREA* GERMAR, 1824 (CHRYSEMELIDAE, LAMPROSOMATINAE) FEEDING ON *PSIDIUM CATTLEIANUM*, SABINE, 1821 (MYRTACEAE)

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In 1991 an agreement between "Universidade Federal do Paraná" and Hawaii University, Manoa, Hawaii, was established in order to recognize the potencial associates insects for the biological control on *Psidium cattleianum* Sabine, 1821 (Myrtaceae).

Lamprosoma azurea Germar, 1824 has been found on this plant in Curitiba, Parana State, Brazil, feeding on the apical parts, causing serious damage. The egg, larvae and adult are described and illustrated. Some biological aspects of this species are provided.

20-196

GENETIC DIVERSITY IN LIFE HISTORY TRAITS BETWEEN POPULATIONS OF THE *DROSOPHILA* PARASITOID, *LEPTOPILINA HETEROTOMA* (HYMENOPTERA: EUCOILIDAE).

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Parasitoid insects are exposed to particular selective constraints because the larval stages live as parasites whereas adults live freely. Thus, a close adaptation is expected in response to variations of their environment.

The species *Leptopilina heterotoma*, a parasitoid of *Drosophila* larvae, is widespread in Europe and shows important variations in life history characters according to geographic origin. Parasitoids originating from mediterranean area show a high rate of locomotor activity, a bimodal circadian rhythm and a high parasitization efficiency. On the other hand, parasitoids collected more in the north have an unimodal rhythm with a low rate of activity and a parasitization efficiency twice less important. Crosses between strains have demonstrated the genetic basis of these variations thus suggesting a local adaptation to selective pressures of the environment.

The geographic differences among populations of *L. heterotoma* could be correlated with climatic latitudinal variations. Furthermore they are also correlated with the presence of *L. boulandi*, the main competitor of *L. heterotoma* in the mediterranean area. The selective role of this interspecific competition is strongly suggested by the clear shift in the phase of circadian activity rhythms of both species which results in a temporal sharing of hosts.

Current studies are trying to evaluate the contribution of the different selective constraints emanating from abiotic environment and from the whole complex of *Drosophila* associated parasitoids.

20-195

CHARACTERIZATION OF A TERATOCYTE-SPECIFIC 540 KDA PROTEIN IN THE BRACONID WASP, *PERILITUS COCCINELLAE* SCHRANK (HYMENOPTERA: BRACONIDAE)

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Perilitus teratocytes became hypertrophic to such an extent that some teratocytes attained a maximum diameter of 820 µm. The number of teratocytes, however, decreased gradually and few or no teratocytes remained in the host body cavity after the parasitoid larva egressed. Changes of the total protein content of teratocytes and parasitoid larvae indicated that the teratocytes may have a role in providing nutrients for the developing parasitoid larvae. This hypothesis was supported by the fact that the gut content of the parasitoid larvae showed an immunoreaction to antibodies against crude teratocyte proteins. We further characterized the 540 kDa teratocyte-specific protein and discussed the function of the *Perilitus* teratocytes.

20-197

MICROSATELLITE AND GENETIC DIVERSITY IN THE ASEXUAL PARASITOID, *VENTURIA CANESCENS*.

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Venturia canescens is an obligate parthenogenetic solitary endoparasitoid of phytophagous moth larvae. The parthenogenesis is not induced by *Wolbachia* or microsporidians and is not cured by antibiotics or heat treatment. No detectable genetic diversity could be detected by alloenzyme studies within or between isolates or clones, but the mechanism of diploid restoration is not certain.

We have cloned, sequenced and characterised over 670 microsatellites from *Venturia canescens*. The genome displays a strong bias to dinucleotide repeats with very few tri- or tetra-nucleotide repeat motifs. Additionally, almost all the microsatellites are small (n<10) and most contain inserts and thus are stable, with no detected polymorphism between isolates.

However, utilising PCR primers to the loci-specific microsatellites we have probed for the genetic diversity between different geographical isolates, between established clones within an isolate, and within the progeny of a single clone of *Venturia canescens*. The data are presented and applications to the behavioural, sexual and evolutionary biology of *Venturia canescens* are discussed and illustrated.

20-198

AN EXPERIMENTAL AND THEORETICAL MODEL TO EXPLAIN SEX-DETERMINATION IN THE ENDOPARASITOID *DIADROMUS PULCHELLUS* (ICHNEUMONIDAE) : VALIDATION OF THE ONE-LOCUS MODEL.

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The competition of endoparasitoid larvae associated with superparasitism has different consequences whether the wasps are gregarious or solitary. However in both case, the ratio of haploid versus diploid wasps produced is modified and the adaptative value of the population is increased.

We have studied the proportion of the eggs giving rise to adults in the solitary wasp *Diadromus pulchellus* (Ichneumonidae). First, taking into account the production of both haploid and diploid males by the females, we have measured the viability of eggs, larvae and pupae without larval competition with the "egg selection coefficient" and the "larval-nymphal selection coefficient".

The study of this coefficient in our experimental population shows four distinct groups of females according to the sex-ratio of their offsprings. These experimental groups are those expected by the theoretical transmission of sexual alleles under the one locus model.

The results show that the probability to develop into an adult that in the laboratory population is higher for a female egg (diploid) than for a male (haploid or diploid).

20-200

Lesser Wax moth, *Achroia grisella* Fab. and its Parasitic Wasps.

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Lesser Wax moth, *Achroia grisella* is one of the important Pests of honeybee, destroying wax combs mainly in the storage. life cycle when reared on old wax comb under 29-31 temp. and 65-75% R.H. decreased the duration of larval period.

In an investigation for number of larval instars, it has been found.

Two species of Parasitoides have been Collected. They are *Dibrachys boarmiae* and *Apanteles galleriae*. This is the first report on this insects in IRAN.

20-199

PARASITISM BY *METEORUS RUBENS* ON *AGROTIS IPSILON* AS AFFECTED BY SUPPLEMENTARY FOOD AND KAIROMONE, FIELD STUDIES.

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Field experiments in clover field showed that releasing of 10 mated *M. rubens* females/m², increased parasitism among *A. epsilon* larvae from 16.7% at 0-day to 43.3% after 10 days. Also, releasing 10 females/m² after spraying 3% molasses in tomato field increased parasitism from 4% at 0-day to 27.5% after 10 days among *A. epsilon* larvae. Spraying molasses and kairomone together on maize plants, increased markedly the rate of parasitism among *S. cretica* larvae comparing with parasitism obtained when each of them was sprayed alone. The release of *M. rubens* in a rate of 10 females/m² after spraying molasses and kairomone induced a significant increase in the rate of parasitism among *S. cretica* larvae after 10 and 15 days compared to releasing 3 or 4 females of the parasitoid.

A significant increase in parasitism among *S. cretica* larvae on tomato after 10 and 15 days was recorded when *M. rubens* was released at the rates of 5 and 10 females/m² after spraying molasses and kairomone, compared to other treatments.

20-201

Some Hymenopterous parasitoids and their potentialities on controlling alfalfa aphids in Karaj, Iran.
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Two alfalfa aphid species, *Acyrtosiphon pisum* (harris) and *Therioaphis trifolij* forma maculata (Buckton) are the economic importance pests on alfalfa in Iran.

In order to investigate the identification and the effect of some hymenopterous parasitoids, sampling of aphids has been done weekly during the spring and summer randomly from different parts of experiment field (one hectare) by stem counting method. Aphids were transferred to lab. The aphids were kept in petri-dishes under 20-22 °C, 60-70% humidity and daylight 16 hours conditions. The adult wasps emerged from aphid mummies and were counted and the percent of parasitism was calculated.

For confirmation, the adult wasps were sent to California (University of Davis)

The wasps were identified as *Aphidius ervi*,

on green alfalfa aphid and *praon palitans* on the spotted alfalfa aphid.

Our investigations showed that, the parasitism of *Aphidius ervi* on green alfalfa aphid has been 9-11% and the parasitism of *praon palitans* on spotted alfalfa aphid has been 12-20%.

20-202

TAXONOMY AND BIOLOGY OF THE THRIPS PARASITOID GENUS *CERANISUS* WALKER (HYMENOPTERA: EULOPHIDAE)

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Recent interest in biological control of thrips pests (Thysanoptera) brought to light several problems associated with the thrips-attacking Entedoninae (Eulophidae). The taxonomy of this genus is in a very poor state worldwide. The extremely small size and rarity of collection of these parasitoids has contributed to the lack of taxonomic activity and makes their identification by current workers difficult. Our objective is to clarify species issues in the genus *Ceranisus* Walker, examine the type material, and provide a key to the world species of *Ceranisus* and other noteworthy entedonines which are known to attack immature stages of thrips.

A review of the Nearctic species of *Ceranisus* has been completed (in press, Transactions of the American Entomological Society). One new species, *C. loomansi* S. Triapitsyn & Headrick, was described. Four other species of *Ceranisus* are redescribed and illustrated based mainly on a study of their type specimens as well as on additional material from the USA and Japan. Lectotypes are designated for *C. americensis* (Girault), *C. nubilipennis* (Williams) and *C. russelli* (Crawford). A key to eight species belonging to four entedonine thrips-attacking genera is given, and host associations are indicated for those species.

20-204

EFFECTS OF LINEAR FURANOCOUMARINS FROM CELERY ON TWO HOST-PARASITOID SYSTEMS: *TRICHOPLUSIA NI* - *COPIDOSOMA FLORIDANUM* AND *SPODOPTERA EXIGUA* - *ARCHYTAS MARMORATUS*

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Experiments were conducted to determine the impact of photoactivated linear furanocoumarins, present in celery (*Apium* spp.) and related plants on two host-parasitoid systems. We compared the development, size and survival of parasitoids reared from hosts fed diets containing linear furanocoumarins with those of unparasitized herbivores. One system consisted of the polyembryonic egg-larval parasitoid, *Copidosoma floridanum* (Ashmead) (Hymenoptera: Encyrtidae), reared from *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). The second system consisted of the solitary larval-pupal parasitoid, *Archytas marmoratus* (Townsend) (Diptera: Tachinidae) reared from *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Increasing dietary concentrations of linear furanocoumarins prolonged larval development of *T. ni* but did not affect *T. ni* size or survival. However, increasing concentrations of furanocoumarins in the host's diet not only delayed development of *C. floridanum* but increased parasitoid mortality. In contrast to *T. ni*, increasing dietary concentrations of linear furanocoumarins increased the mortality of *S. exigua*. However, furanocoumarin concentration did not affect the size of surviving *S. exigua*. Increasing concentrations of furanocoumarins in the diet of *S. exigua* resulted in increased mortality of *A. marmoratus*. Similar to the effects on *S. exigua*, increasing concentrations of furanocoumarins did not affect the size of surviving *A. marmoratus*. Despite the prolonged larval development of *S. exigua*, there was no effect on *A. marmoratus* development time following host pupation.

These results suggest linear furanocoumarins have differential impacts on the two systems. The linear furanocoumarins did not affect polyembryonic development of *C. floridanum*, but were toxic to developing immatures. The linear furanocoumarins did not appear to be directly detrimental to *A. marmoratus*, but their impact was mediated through stresses to the host, *S. exigua*.

20-203

SEASONAL ABUNDANCE OF THE CITRUS LEAFMINER AND ITS PARASITOIDS IN FLORIDA CITRUS

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Seasonal abundance and parasitism of the citrus leafminer *Phyllocnistis citrella* Stainton, were investigated from summer 1993 through December 1995 on "Tahiti" limes in Florida. Citrus leafminer densities increased from spring through fall and declined during winter 1994 and 1995. Eight species of parasitoids attacked citrus leafminer immatures in commercial and experimental orchards. The eulophid *Pnigalio minio* (Walker), a primary ectoparasitoid of dipterous and lepidopterous leafminers, comprised about 80% of the parasitoids which emerged from *P. citrella*. Species and abundance of adult parasitoids varied considerably between leafminer generations. Based on the examination of 15,000 larvae and pupae of the leafminer, these parasitoids are apparently everywhere throughout the region, and parasitization rates exceeding 50 % have been observed in unsprayed groves. In general a positive correlation between leafminer populations and parasitoid densities demonstrated that indigenous parasitoids significantly reduced leafminer densities 2.5 years after its invasion of Florida.

20-205

THE TRENDS OF DIAMONDBACK MOTH PARASITOIDS ON CABBAGE IN RELATION TO DIFFERENT MIXED CROPS

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Diamondback moth (DBM), *Plutella xylostella* (L.) is the most important pest on cabbage in North Vietnam. The population fluctuations and abundance of the pest were investigated weekly on cabbage (*Brassica oleracea* L., *B. gongilodes* L.) from November to March of two years 1992-1994. The investigations carried out in two localities of Red River Delta showed that of two important parasitoids reared from DBM, the pest pupae parasitized by the pupal parasitoid *Phaeogenes* sp. (Ichneumonidae) was very low (maximum 3.3%) in comparison with the sole larval parasitoid *Cotesia* (= *Apanteles*) *plutellae* Kurdj. (Braconidae) (average 30.8%).

It was found that cabbage fields adjacent to maize and rice fields often have significantly lower parasitized pest population by *C. Plutellae* than those on cabbage intermixed with different crops of Solanaceae, Fabaceae groups and some other decorative flowers (Asteraceae), for the maximal proportion of parasitized pest larvae was less than 25% (average 13.1%) on cabbage grown alone compared to percentage of parasitism up to 40% (average 17.7%) on the mixed planting cabbage with different crops.

In addition, cabbage fields of both above localities often treated by some pesticides as Cartap (PADAN 95BHN), Phethoate+Enthofenprox (CIDI M) but it was observed that the peak of parasitoid populations usually occurred in the middle and towards the end of the crop.

20-206

PARASITOIDS OF FRUIT-INFESTING TEPHRITIDAE—HOW TO ATTACK A CONCEALED HOST

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Fruit-infesting Tephritidae, exemplified by such economically important genera as *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus*, and *Rhagoletis*, are remarkably diverse with over 1400 species world-wide attacking fleshy fruits. They, in turn, are attacked by a wide variety of parasitic Hymenoptera, including members of the families Braconidae, Chalcididae, Diapriidae, Eucilidae, Eulophidae and Pteromalidae. Although some are generalists, most species appear to be specific on Tephritidae. Further, extensive rearings of Tephritidae from fleshy fruits and from flower heads suggests that there is no overlap among the parasitoids attacking hosts in these two habitats. Among the opiine Braconidae, the most diverse group of parasitoids attacking fruit-infesting Tephritidae, there is a high degree of such habitat specificity even at the generic level. All known species of *Fopius* and *Diachasmimorpha*, for example, attack fruit-infesting Tephritidae. Interesting exceptions in the genera *Psytalia* and *Utes* are discussed. Knowledge of these relationships, and how they have evolved, is of particular value in predicting what types of host shifts can occur following importation for biological control.

Tephritid parasitoids exploit their hosts in a variety of ways. Egg-larval parasitism, with emergence from the host puparium, has been well-studied in *Fopius arisanus*, but occurs in several other opiines as well. The remaining opiines and all eucilids attack various larval instars, emerging from the puparium. Most of these oviposit through the fleshy portion of the fruit. At least some tetrastichine eulophids, however, attack the host directly, either by penetrating the fruit or by ovipositing on larvae as the latter emerge from fruit to pupate. Most fruit-infesting tephritids pupate in the soil, where they are subject to attack by chalcids and diapriids uniquely adapted for subterranean exploration. Though relatively few species have been studied in detail, critical examination of morphological features and a sound phylogenetic perspective should enable us to predict the attack strategies of most tephritid parasitoids.

20-208

EFFECT OF DIFFERENT COMBINATIONS OF TEMPERATURE ON THE REPRODUCTION OF *SPALANGIA GEMINA* BOUCEK (HYMENOPTERA, PTEROMALIDAE), PUPAL PARASITOID OF *MUSCA DOMESTICA* L. (DIPTERA, MUSCIDAE)V.A. Costa¹, E. Berti-Filho¹, J.R.P. Parra¹¹. Departamento de Entomologia-ESALQ/USP, Piracicaba, SP, Brazil

Spalangia gemina Boucek is one of the main parasitoids attacking pupae of *Musca domestica* L. and other synanthropic flies in poultry manure in the State of São Paulo, Brazil. This paper deals with the effect of different combinations of temperature on the immature and adult stages of *S. gemina* regarding the reproduction of this parasitoid on *M. domestica* pupae. The temperatures tested were 20, 25 and 30°C, 70 ± 10% relative humidity and 14 hour photoperiod. The temperature at which occurred the development of *S. gemina* did not affect the longevity of the females, but did influence their reproduction. Females from immatures reared at 30°C parasitized a lower number of *M. domestica* pupae and consequently produced lower progeny. The parasitism was more effective with females at 25°C, regardless the temperature conditions in which the immatures were reared.

20-207

MORPHOLOGICAL DEFENSE OF WHITEFLY NYMPHS AGAINST NATURAL ENEMIES

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The effect of whitefly nymph's morphological features in the ecological context and especially their defensive role against natural enemies were investigated. Two whitefly species, *Bemisia tabaci* and *Aleyrodes singularis*, were chosen. *B. tabaci*, which is a major agricultural pest, also exhibits intraspecific, environmentally dependent, phenotypic variation (setose vs. smooth nymphs), which could be tested for its adaptive value for defense against predators. *A. singularis*, actively changes its innate phenotype by piling up on the nymphal dorsum exuviae and wax secretion. By removing these additions we could test for their possible defensive value when confronted with parasitoids and predators.

Significant effects of the different phenotypes on the interaction with natural enemies were found in both species. In the case of *B. tabaci*, a defensive effect was achieved when the setose phenotype developed on tomentose leaves. Indeed, we found that leaf tomentosity induces the setose phenotype. In *A. singularis*, the maximal defensive effect was achieved when both exuviae and the ovipositing mother were present on the patch of the developing whitefly nymphs. The mother continued to produce and spread wax upon the developing nymphs until they reached adulthood, a behavior that can be interpreted as parental care.

20-209

SURVIVAL OF THE FIRST AND SECOND COMERS IN SELF-SUPERPARASITISM AND CONSPECIFIC SUPERPARASITISM

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Using *Haplogonatopus atratus* (Hymenoptera: Dryinidae), a solitary parasitoid of the small brown planthopper, *Laodelphax striatellus* (Hemiptera: Delphacidae), we reveal the survival rates of the first-laid and second-laid offspring in superparasitism. Although it is usually impossible to distinguish between the first comer and the second comer in superparasitism, it is possible in this parasitoid because the immature parasitoid remains adherent to the oviposition point. Recording of the oviposition point and everyday observation of the superparasitized host clarifies the fate of the first comer and that of the second each. A healthy 4th-instar larva was parasitized within one day after molting. Then that larva was superparasitized 1, 2, 4, 8, 12, 24, 48, 72, or 96 hours later. Unmated parasitoid females were used for the experiment, i.e., eggs laid were all male, because there is a difference in the survival rate of the immature between the male and the female.

The survival rate of the first comer increased with increase of interval of the first and second ovipositions, while the survival rate of the second comer decreased, and consequently the survival rates of the two became similar for oviposition intervals of more than 1 day. For almost all intervals the survival rates of the first and second comers were each higher in conspecific superparasitism than in self-superparasitism. As a result, the proportion of hosts producing parasitoid adults was higher than that in solitary parasitism (60.0%) or almost the same, while in self-superparasitism the proportion was similar to the survival rate in solitary superparasitism or less than that.

20-210

INFLUENCE OF WHEAT AND OAT CULTIVARS ON THE DEVELOPMENT OF CEREAL APHID PARASITOIDS (HYMENOPTERA: BRACONIDAE)

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Wheat and oat cultivars with different levels of Hydroxamic acids, secondary metabolites involved in plant resistance against cereal aphids, were used to evaluate the influence of these compounds on the development of specialist and generalist aphid parasitoids attacking the host *Sitobion avenae* (Fabr.).

Higher Hx levels caused a significant increase in developmental time of the oligophagous parasitoid *Aphidius rhopalosiphii* De Steph., which is mainly accounted for by an increase in the parasitoid prepupal developmental time. However, the polyphagous *Ephedrus plagiator* (Nees) was not affected by Hx levels.

20-2012

THE BIOLOGY OF *ANAGYRUS DACTYLOPII* (HOW), (HYMENOPTERA: ENCYRTIDAE) ON *NIPAECOCCLUS VIRIDIS* (NEWSTEAD), (HOMO.: PSEUDOCOCCIDAE) IN SOUTHWEST IRAN.

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The mealybug, *Nipaecoccus viridis* (Niwstead) is one of the major pest of *citrus* spp. In Khuzestan southwest of Iran. The encyrtid parasitoid, *Anagyrus dactylopii* (How), parasitize up to 40% of the mealybug under the natural conditions. In a survey during 1994-95 the biological aspects as well as parasitoid/host interaction between *A. dactylopii* and *N. viridis* were studied at 26±2 C., 65±5%RH, and under a photoperiod of LD 14:12. The mealybugs were reared on pumpkin and sprouted potato tubers under the same conditions. *A. dactylopii* oviposits in all there nymphal and in the adult female stages of *N. viridis* when each stage was exposed separately. However, the frequency of hosts parasitized, and number of eggs deposited per host were significantly greater in third nymphal and adult female mealybugs than in either first or second stage nymphs. The sex ratio of female to male parasitoids was also the highest following oviposition in third nymphal and adult female host. The total developmental time (egg to adult) for male and female parasitoid were 12-15 ($\bar{X}=13.35\pm0.86$) and 13-16 ($\bar{X}=14.35\pm0.85$) days respectively. The developmental time of eggs, larvae and adults as well as sex ratio on three nymphals and adult stages were determined.

20-211

BIOLOGICAL CONTROL OF THE BLUE GUM PSYLLID, *CTENARYTAINAE EUCALYPTI* (MASKELL) (HOMOPTERA: PSYLLIDAE), IN CALIFORNIADahlsten, D. L., D. L. Rowney, W. A. Copper, R. L. Tassan, W. E. Chaney¹, K. L. Robb², S. Tjosvold³, M. Bianchi⁴ and P. Lane⁵

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The blue gum psyllid was first discovered in Monterey County, California, USA in January 1991. Since the original find the blue gum psyllid has spread in a very short time throughout the California coastal area, and has become a major pest on *Eucalyptus pulverulenta* foliage plantations. Large amounts of pesticides have been used to control the psyllid in these plantations. One species of primary parasitoid wasp, *Psyllaephagus pilosus* Noyes (Encyrtidae) was found in Australia and New Zealand, and released at eight sites in California in spring/summer 1993. Psyllid populations declined somewhat at most sites in 1993 compared to 1992; in 1994 and 1995 psyllid populations had declined several hundred times below the pre-release levels, and were no longer a problem at all sites where parasitoid releases were made. The parasitoid has spread rapidly to other coastal areas and has controlled the psyllid levels in these areas in 1995.

20-213

HOST-FEEDING BEHAVIOR AND MATERNAL CARE IN *CEPHALONOMIA STEPHANODERIS* (HYMENOPTERA: BETHYLIDAE), AN ECTOPARASITOID OF THE COFFEE BERRY BORER.

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Cephalonomia stephanoderis Betrem is an ectoparasitoid of the prepupae and pupae of *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), the most important pest of coffee worldwide. Although some studies have been done on its biology, the essential aspects of the behavior of this parasitoid have been neglected. In this work the host-feeding behavior and the influence of maternal care on brood survivorship were studied under laboratory conditions.

C. stephanoderis females remain with their brood after oviposition. Experimental removal of the mother influenced larval fitness: survival of broods guarded by the parental wasp was higher than that of broods without the attending mother (75% vs 45%). Moreover, most of larvae not attended by the mother failed to built a cocoon and the mortality of these naked pupae was higher (33% vs 15%). The behavior of the attending females was, nevertheless, influenced by their physiological state: starved females attacked their own eggs or larvae but the formation of a cocoon prevented the pupae from being fed.

By studying the influence of food on oögenesis, we were able to show that females which had not consumed a proteinaceous meal from the host body fluids were not able to mature eggs. Egg load (number of mature and developing oocytes present in the ovary of the female) influenced the oviposition behavior of *C. stephanoderis* females but had no influence on their feeding behavior. Host-feeding in *C. stephanoderis* is non-concurrent, wasps destroy the hosts from which they feed. Both the parasitic and predatory behaviors of *C. stephanoderis* females may be exploited to control *H. hampei*. This enhances the potential of this species as a biological control agent.

20-214

BIOLOGICAL CONTROL PROGRAM FOR AN INTRODUCED MEALYBUG, *ORACELLA ACUTA* (LOBDELL) (HOMOPTERA: PSEUDOCOCCIDAE), IN THE PEOPLE'S REPUBLIC OF CHINA

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The mealybug *Oracella acuta* was introduced into Guangdong Province in the People's Republic of China (PRC) from the United States in 1988. Fifty hectares of slash pine forest were infested in 1990, increasing to 220,000 ha in 1995. The rate of spread is approximately 17-22 kilometers/year. Severe infestations are currently in exotic slash and loblolly pine plantations, but native Mason pine is also threatened. Pines are not killed, but growth and yield are reduced.

Populations in the U.S. are normally regulated at low levels by natural enemies, so a joint biological control project was initiated between the PRC and the U.S. to introduce natural enemies of *O. acuta* into the PRC. Parasitoids, including *Allotropa* sp. and *Acerophagus coccis* E. Smith, have been reared from greenhouse and field infestations of *O. acuta* in the southeast U.S. and shipped to Guangdong Province. The Chinese have released some parasitoids, and they are currently monitoring their establishment.

20-216

LARVAL AND PUPAL PARASITOIDS OF THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) AND THEIR SEASONAL OCCURRENCE IN CABBAGE FIELDS IN NORTH JAPAN.

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Seven primary parasitoids, three facultative secondary parasitoids, and seven secondary parasitoids were recorded from larvae and pupae of the diamondback moth *Plutella xylostella* in cabbage fields in North Japan. Among the primary parasitoids, *Cotesia plutellae*, *Diadegma niponica*, *Diadromus subtilicornis*, and *Oomyzus sokolowskii* were dominant. *O. sokolowskii* was also the parasitoid of *C. plutellae*, i.e. the secondary parasitoid of *P. xylostella*.

The density of 4th-instar larvae of *P. xylostella* per plant started to increase in late June in 1994 and 1995, and abruptly decreased in early August after showing a peak in July. The occurrence of the pupae showed the same pattern with several days delay. The parasitism of the 4th-instar larvae by *C. plutellae* and *D. niponica* was observed as early as in late May, but *D. niponica* disappeared in middle July. The parasitism of the 4th-instar larvae by *C. plutellae* and *O. sokolowskii* and that of the pupae by *D. subtilicornis* increased in late July when the host density decreased. The parasitism in the pupae continued to be more than 40% from early August to middle November, so that the density of *P. xylostella* seemed to be kept low after August.

20-215

INFLUENCE OF THE DEFENSIVE BEHAVIOUR OF THE CITRUS BROWN APHID, *TOXOPTERA CITRICIDUS* (KIRKALDY) (HOMOPTERA: APHIDIDAE) ON THE PRECISENESS OF THE HOST DISCRIMINATION BY ITS PARASITOID, *LYSIPHLEBUS JAPONICUS* ASHMEAD (HYMENOPTERA: APHIDIIDAE).

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The brown citrus aphid *Toxoptera citricidus* (Kirkaldy) jerks up and swivels its abdomen when it is attacked by an Aphidiid parasitoid *Lysiphlebus japonicus* Ashmead. Once the swiveling was initiated in a part of a colony, it could quickly propagate the same synchronous movement among other individuals which had not been attacked by the parasitoid. The effect of this group defense on the oviposition success of the parasitoid was more obvious in older aphids. When a parasitoid allowed to attack in a colony consisting of each ten 1-day-old parasitized (1 hour before) and unparasitized aphids, it could precisely discriminate unparasitized hosts from parasitized ones. It was observed that the parasitoid used both external (by drumming) and internal (by light stabbing) markers in the course of host discrimination. On the contrary, when the same experiment was undertaken using 5-day-old (more defensible) aphids, the frequency of superparasitisms by the parasitoid increased significantly. Since no difference is expected in the outcome of intra-specific competition of the parasitoid larvae between the two host ages, the high frequency of superparasitisms in the colony of 5-day-old aphids seemed not to be ascribed to parasitoids' decision but to be the result from stronger disturbance effect of group swiveling on the discrimination process of the parasitoid.

20-217

A. *CLAVICORNIS*, A STEPPING STONE IN THE EVOLUTION OF PARASITOID BEHAVIOR IN THE ROVE BEETLE GENUS *ALEOCHARA* (COLEOPTERA: STAPHYLINIDAE)

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Larvae of the genus *Aleochara* live as solitary ectoparasitoids on pupae of cyclorrhaphous diptera within the puparium. Morphological and behavioral adaptations to host finding, orientation on the host, gnawing an entrance hole into the puparium shell, avoidance of competition, site and mode of pupation have been comparatively investigated in numerous species of different subgenera.

A. (Heterochara) clavicornis has free roaming larvae with quite different morphology (all campodeoid in contrast to eruciform L2 and L3 in other *Aleochara* species). The predaceous larvae of are capable to overwhelm migrating blow fly maggots and to feed upon unupated and pupated hosts as well. They are also able to move between hosts of different stage and occasionally feed in groups.

Evolutionary tendencies in the parasitoid life habit and the phylogeny of the genus are discussed.

20-218

BIOLOGICAL CONTROL OF THE CHESTNUT GALL WASP IN JAPAN UTILIZING A PARASITOID IMPORTED FROM CHINA (HYMENOPTERA: CYNIPIDAE-TORYMIDAE)

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As a biological control agent of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, a parasitoid, *Torymus (Syntomaspis) sinensis* Kamijo was imported from China, and it was released in Tsukuba, Ibaraki, central Japan, and Ohzu, Kumamoto, southwestern Japan in 1982. After establishment, the parasitoid population increased, and the host cynipid galls decreased under EIL by 1988 in Ibaraki. While, in Kumamoto, the parasitoid population had not increased in six years following its release but has increased since 1989, reaching a level of 28 % in parasitism in 1995. Two factors might play an important role in the delay in the increment of the parasitoid; the low female sex ratio during several years after establishment, and high mortality associated with the activity of native facultative hyperparasitoids.

20-219

EFFECTS OF PUPAL EXTRACT OF *GALLERIA MELLONELLA* ON DEVELOPMENT OF AN ENDOPARASITOID, *VENTURIA CANESCENS* (GRAVENHORST) (HYMENOPTERA: ICHNEUMONIDAE)

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Venturia canescens was reared from egg to pupa on a semi-artificial diet containing pupal extract of *Galleria mellonella*. *G. mellonella* pupae were heated at 60 °C for 10 min and homogenized with the same amount of MGM-450 insect cell culture medium. After centrifugation, the supernatant was sterilized by filtration, and used as a diet. Diets were prepared from the pupae of 0, 1, 2 and 3-day old. On all the diets, 48 h-old *V. canescens* eggs developed to the last instar (5th instar). The extract from 1-day old pupae was the most effective, and supported the development of 90 % of larvae to the last instar, however, none of the larvae became pupae unless the diet was frequently renewed, and obtained pupae did not emerge. Pre-germ band stage-eggs of the parasitoid, within 4 h after oviposition, also developed successfully on this diet and 2 out of 62 pupated. The growth promoting ability of *G. mellonella* extract was lost by heat treatment at 80 °C for 10 min or by passing it through molecular sieve of 30,000 or 100,000 M.W.

20-220

FIELD EVALUATION OF IN VITRO REARED *CATOLACCUS GRANDIS* (HYMENOPTERA: PTEROMALIDAE) AS A BIOLOGICAL CONTROL AGENT OF THE BOLL WEEVIL (COLEOPTERA: CURCULIONIDAE)

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The efficacy as a biological control agent of the ectoparasitoid *Catolaccus grandis* (Burks) females reared in a solid artificial diet was evaluated in South Texas. For the first time in the United States, field releases of an in vitro reared parasitoid were done using a diet formulated without the use of insect components. Percent parasitism observed in the field during May, 1994 was 55.6, 57.3, and 39.4% during the second, third, and fourth weeks of releases, respectively.

The searching capacity and dispersal ability of in vitro reared *C. grandis* were compared with in vivo reared parasitoids under field conditions in South Texas in 1995. There was not significant difference in the incidence of parasitism inflicted by in vitro and in vivo reared *C. grandis* to boll weevil larvae distributed within a 30m and 60m radius from the release point. Boll weevil life tables calculated from cohorts placed within 15 and 45m from the release point showed no significant differences in mortality induced by parasitism by in vitro and in vivo reared *C. grandis*. These results indicate that the use of artificial diets to mass produce quality *C. grandis* is promising.

20-221

PARASITIDS OF *HELICOVERPA ARMIGERA* (HBN.) (LEPIDOPTERA: NOCTUIDAE) ON GREENHOUSE TOMATO CROPS IN THE OESTE REGION OF PORTUGAL. A PRELIMINARY STUDY.

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As a result of larval collection of tomato fruitworm, *Helicoverpa armigera* (Hbn.), on greenhouse tomato crops in order to diagnose eventual insect diseases and to obtain larvae for laboratory rearing, high levels of parasitism were recorded in greenhouses under IPM schemes, in some cases almost controlling the pest, in contrast to greenhouses with traditional chemical pest management.

The parasitoid complex was determined and compared with other references of parasitism of the tomato fruitworm, in greenhouse and outdoors crops, in Iberian Peninsula and northern Africa to discuss its importance. The role of this parasitism in greenhouse IPM programs, in the Oeste and in other regions, is also evaluated.

20-222

DIGLYPHUS POPPOEA WALKER (HYM: EULOPH.) AS AN IMPORTANT NATURAL CONTROL AGENT OF *LIRIOMYZA HUIDOBRENSIS* (BLANCHARD) (DIPT.: AGROMYZIDAE) IN PORTUGAL

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Recent studies on leafminers in Portugal, conducted in some greenhouses in the Oeste region, approximately 40-50 Km north of Lisbon, allowed the characterization and evaluation of the leafminers parasitoids population.

The hymenopterous parasitoids were reared from *Liriomyza huidobrensis* (Blanchard) larvae sampled from greenbeans, lettuce, tomato and pepper.

In 1993 about 39% of the samples revealed the presence of *Diglyphus poppoea* Walker. During 1994 and 1995 this species had similar representativity and was present all over the year. The relative importance as natural control agent is presented and its abundance is discussed, taking into account the differences between the greenhouses where the samples were taken.

20-224

DISTRIBUTION AND DORMANCY OF THE PARASITIDS ATTACKING *CHROMATOMYIA FUSCULA* (ZETTERSTEDT) (DIPTERA : AGROMYZIDAE) IN NORWAY

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The larvae of *Chromatomyia fuscula* mine the leaves of cereals and other grasses, and the species is a cereal pest in parts of Norway. It pupates in the leaf, and has one generation per year (adults overwintering). It is attacked by chalcid (Eulophidae and Pteromalidae) and braconid (Braconidae) parasitoids.

In July - Sept. 1994, barley leaves with mines were collected from 18 fields, ranging from 58 to 69.5°N and from 5 to 540 m above sea level. The parasitoids emerging from the leaves were identified and counted, followed by an inspection of each mine. The number of species in a sample of 45 leaves ranged from 1 to 9 (mean=4.6) and decreased with the latitude and increased with the number of specimens (mean number=48). The most numerous parasitoid was *Cyrtogaster vulgaris* (Walker) (Pteromalidae), a pupal parasitoid overwintering as an adult. It was not found in the three highest (>300m), nor in the three northernmost (>65°) localities.

The mine inspection revealed dormant parasitoid larvae in 80% of the localities north of 60° (N=11). These larvae were kept at about 5°C over winter, and adults of at least 6 different species, both larval and pupal parasitoids, emerged the following spring. Further south these species emerge in the late summer. North of 60° they seem to have a mixed strategy (i.e. they were found both as newly emerged adults and as larvae in the autumn under lab conditions).

20-223

SYNTHESIS OF A TERATOCYTE-SPECIFIC PROTEIN IN THE BRACONID WASP, *PERILITUS COCCINELLAE* SCHRANK (HYMENOPTERA: BRACONIDAE)

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During parasitism, *Perilitus* teratocytes became hypertrophic and enlarged about ten-fold in diameter from 50 to 500 µm on average. The increase in protein content of the teratocytes mirrored the increase in protein synthesis rate. Non-denaturing-PAGE revealed that the teratocytes contained a major polypeptide with a high molecular weight of about 540 kDa. The teratocytes were cultured in vitro in a medium containing ³⁵S-methionine. Both the proteins from the incubated teratocytes and the medium were subjected to PAGE followed by fluorography. The fluorogram revealed that the teratocytes synthesized the 540 kDa protein. The teratocytes had a tendency to accumulate the synthesized teratocyte proteins without release. We clarified the possibility of the active secretion by a pulse-chase experiment. Finally, we discuss a possible function of the teratocytes in *P. coccinellae*.

20-225

MALAISE CATCHES OF CHALCID PARASITIDS OF THE LEAFMINER *CHROMATOMYIA FUSCULA* (ZETTERSTEDT) (DIPTERA: AGROMYZIDAE) DURING FOUR YEARS IN AN ORGANIC BARLEY FIELD AND ITS SURROUNDING VEGETATION.

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The leafminer *Chromatomyia fuscula* is an annual pest in cereals and grasses in Norway. Malaise traps were placed in an organically managed barley field and in the surrounding vegetation at Ås, southern Norway, in 1992-95. The chalcid parasitoid species which we previously had reared from *C. fuscula* on barley were sorted out.

The total number of chalcid parasitoid specimens in the traps varied considerably from year to year. Each year the traps collected about 9-14 of the ca 15 species totally reared from the leafminer. The dominant species alternated between the pupal parasitoid *Cyrtogaster vulgaris* (Walker) (1993, 1995) and the larval parasitoid *Diglyphus begini* (Ashmead) (1992 in the field, 1994). *D. begini* had a higher relative frequency in the field compared to the edge area, whereas the opposite was the case for *C. vulgaris*. Each year, at least one of these two species also dominated the parasitoid complex that emerged from barley samples infested with the leafminer.

The phenology and sex ratio are shown for some dominant species.

20-226

INTER- AND INTRASPECIFIC VARIATION IN *EUBAZUS* SPP. (HYMENOPTERA: BRACONIDAE) PARASITOIDS OF *PISSODES* WEEVILS (COLEOPTERA: CURCULIONIDAE)

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Eubazus spp., egg-prepupal parasitoids of *Pissodes* weevils, are considered as potential biological control agents against the white pine weevil, *Pissodes strobi* (Peck), in North America. In Europe, three closely related species exist, with separate microhabitat affinities: *Eubazus semirugosus* (Nees) is a parasitoid of *Pissodes* spp. in pine trunks, *E. robustus* (Ratz.) attacks *P. validirostris* (Sahlberg) in pine cones, and an undescribed species parasitises *P. piceae* (Ill.) in fir trunks. Populations of these species were compared in traits likely to affect establishment or control by biological control agents. Variation between species was found in development time, diapause, host preference, fecundity and functional morphology. Intraspecific variability was observed in diapause requirement between climatic biotypes of *E. semirugosus* and *E. robustus*. Differences observed within, and between *Eubazus* spp. are regarded as an adaptation to the biology of their respective hosts. Implications of these differences in the biological control of *P. strobi* are discussed.

20-228

HOST AGE PREFERENCE AND FUNCTIONAL RESPONSE IN AN ECTO PARASITOID, *HEBROBRACON HEBETOR* SAY (HYMENOPTERA: ICHNEUMONIDAE).

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Through analysis of variance it was observed that parasitoid females paralyzed host larvae indiscriminately when offered *Anagasta kuhniella* (Zeller) larvae of 3 different ages daily for four consecutive days, since emergence. They did not present significant preference in relation to the day of offering ($p = 0.267$) and the size of host larvae ($p = 0.073$). On the contrary, significant differences were observed in average number of larvae parasitized in relation to the day of offering ($p = 0.049$) (2nd and 3rd day) and the age ($p = 3.578 \times 10^{-5}$) (5th instar) of host larvae. These results indicate that the female parasitoids ensure the oviposition sites by paralyzing maximum number of larvae but deposit the eggs preferentially in the larger larvae.

Functional response of *Hebobracon hebetor* say was analyzed at 10 (5, 10, 15, ..., 50) different host densities in relation to 3 distinct behaviors of females: number of larvae paralyzed, number of larvae parasitized and number of eggs deposited. A type I functional response was exhibited by the female parasitoid in response to host density only in case of number of larvae paralyzed. These informations are important for mass-rearing of *H. hebetor*, for biological programs against *A. kuhniella*

20-227

SURVEY OF NATURAL ENEMIES OF *Spodoptera frugiperda* IN THE SOUTH OF BRAZIL¹
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A survey of natural enemies of fall armyworm, *Spodoptera frugiperda*, was done in maize producing region, in South of Brasil, during 1992/93 and 94/95 years. Insects were collected in 5 different places, and in each place 2 different maize crops were selected. In each maize crop about 90 larvae were collected. All larvae were brought to the laboratory, fed with artificial diet and observed daily. Results showed that the parasitoids were basically the same, varying the frequency depending on the place. The same was observed in relation to viruses. *Campoletis grioti* (Hymenoptera: Ichneumonidae) and *Archytas marmoratus* (Diptera: Tachinidae) were the most frequent parasitoids. *Campoletis grioti* sometimes occurred in 47% of the sampled larvae, and *A. marmoratus* in 15% of the sampled larvae. *Eiphosoma vitticollis* (Hymenoptera: Ichneumonidae) was also found parasitizing *S. frugiperda* larvae, but at low levels. *Baculovirus* showed a high natural incidence in some places killing up to 10% of the larvae. Only one granulosis virus was found. The fungi *Nomuraea rileyi* occurred only in one place attacking 10% of the larvae. Some diptera and hymenoptera died during the larval stage and were not identified.

20-229

MICROCHAROPS ANTICARSIAE GUPTA (HYMENOPTERA: ICHNEUMONIDAE) AS A DISSEMINATOR OF ENTOMOPATHOGENIC FUNGUS *NOMURAEA RILEYI* (FARLOW) SAMSON AND ITS EFFECT ON THE DEVELOPMENTAL STAGES OF PARASITOID.

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Under laboratory conditions, when female ovipositors were contaminated manually and with fungus hyphae, they were unable to transmit the infection to host (*Anticarsia gemmatilis* Hüb.) larvae.

Different concentrations of fungus affected the duration time of larval ($F=29.692$; $df=4$; $p<0.01\%$) and pupal ($F=15.335$; $df=4$; $p<0.01\%$) stages. Only high concentration, 1.97×10^6 of viable spores affected the emergence of parasitoid larvae from host ($\chi^2=31.4058$; $P<0.1\%$). To verify the effect of infection on the development of parasitoid larvae; host larvae were first parasitized at different time intervals such that on the day of inoculation 0, 1, 2, 4 and 8 days parasitized larvae were present. Parasitoid cocoon formation did not suffer significant reduction ($\chi^2=3.77$; $df=1$; $p=5.31\%$) only in hosts parasitized 8 days prior to inoculation. Effects of fungus on the developmental stages of parasitoid revealed that *N. rileyi* has a negative effect on parasitism of *M. anticarsiae* in host larvae. Moreover such effects reflect in the suppression of parasitism as well as it delays the development of parasitoid in the host larvae.

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20-230

ADAPTIVE UTILIZATION OF HOST RESOURCE BY EULOPHID PARASITOIDS OF LEAF-MINING FLY

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The leaf-mining larvae, *Phytomyza ranunculi* Schrank, are parasitized by more than ten species of eulophids in Japan. These eulophids parasitize hosts after killing them.

Such a parasitization pattern will lead to the limited supply of resource to their progeny within the host and, in fact, decreased the fitness of their progeny, especially obtained from young hosts; for example its decreased body-size, longevity and fecundity. Eulophid females often sucked host fluid through the small opening produced by their ovipositor in the epidermis which covers the host mine. This always led to the death of hosts. The ratio of killed hosts by host feeding and parasitization was 1:2 to 1:1 on the average through their life. They were unable to live long and to initiate oogenesis without a supply of the energy by host feeding, probably because they allocate most of the limited supply of energy from the killed host toward a construction of their body, so store less energy as an adult. Thus, they supply such a deficiency of saprophaga in their larval stage by an adaptive utilization of hosts as follows.

First, they preferred young hosts with a low quality for host feeding and mature hosts with a high quality for parasitization. Also, they laid preferentially a male egg in young hosts but a female egg in mature hosts.

20-232

OCCURRENCE AND CONTROL OF BEET ARMYWORM, *SPODOPTERA EXIGUA* HUBNER (LEPIDOPTERA: NOCTUIDAE), USING SYNTHETIC SEX PHEROMONE IN KOREAJong Dae Park, Kyu Chin Kim¹

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This study was carried out to investigate seasonal occurrence of the Beet armyworm(BAW) *Spodoptera exigua*, and on suppress of their density collected by mass trapping method and control effect in field using mating disruption dispenser of synthetic sex pheromone in Korea from 1992 to 1994. Adults males of BAW were collected by pheromone from early April through early November. The numbers began to increase abruptly from early July, and forming three peaks on mid. July, early August and early September. In control effects using mass trapping methods, number of leaves damaged by BAW were 0.2-3.0 individuals per 60 hills of *Allium fistulosum* comparing with 33.2 individuals at non-treated field. Also, the number of male adults attracted to the pheromone were 1.8-5.0 individuals at the beginning of installation but increased gradually afterward. There was a negative correlation between amount of male adults attracted to the pheromone and number of damaged leaves($r=-0.9572^*$). Densities in egg and larva of BAW in field treated with mating disruption dispensers was 1.9% level of that without dispensers in welsh onion in 1993. Also, 5.9% and 5.2% were counted from 10ha and 20ha of welsh onion field comparing with fields without dispensers in 1994. Rate of damages in welsh onion fields were 1.6% and 3.0% level in 10ha and 20ha, respectively, and control value were maintained at 80% level until harvest date, October.

20-231

LARVAL PREDATORY BEHAVIOUR OF THE ECTO-PARASITOID *DIGLYPHUS ISAEA* (WALK.) (HYMENOPTERA: EULOPHIDAE)

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Diglyphus isaea (Walk.) is a palearctic parasitoid widely used in Europe for biological control of the nearctic leafminer *Lirionomyza trifolii* (Burg.) (Diptera: Agromyzidae). Adult female paralyzes the host larva before oviposition and lays one egg (exceptionally 2-5) nearby; it is generally described as a 'facultatively gregarious ecto-parasitoid'. Three experiments were carried out using third-instar larvae of *L. trifolii* (infesting bean leaves) as hosts. In the first experiment (n=98), several adult females were placed on a leaf containing one host larva to induce superparasitism. The newly-hatched parasitoid larvae soon started to explore the neighbourhood, killing the other parasitoid eggs or cannibalising each other. Two days later, gregarism was still occurring in 16.3% of hosts, but a maximum of one parasitoid pupated per host. In the second experiment (n=40), over-infested leaves were used with several *L. trifolii* larvae of different ages and wide interconnections of mines. *D. isaea* was allowed to paralyse only one host and lay only one egg. The newly-hatched larvae explored the microhabitat: some of them (30.0%) met an active *L. trifolii* larva and killed it for feeding in 50.0% of meetings. In the third experiment (n=29), some parasitoid larvae cohabited with several host larvae, that could be active, only paralysed (n=139 in total) or parasitized (n=132 in total). Cannibalism was recorded in 30 cases (22.7% of parasitized larvae); predation of active larvae occurred 18 times and *D. isaea* larvae left the original host to move to another paralysed larva in 12 cases. From the first to the third experiment, development time decreased for both sexes, female length increased (1.27 ± 0.37 , 1.33 ± 0.24 , 1.46 ± 0.24 mm respectively; means±ds) and adult emergence (35.7%, 62.5% and 80.3% of parasitized larvae respectively) increased too. These findings indicate that *D. isaea* is a 'solitary ecto-parasitoid' and that the double role of parasitoid-predator within the peculiar microhabitat of a over-infested leaf can improve the fitness of the larvae, also explaining the strong propensity of adult females to paralyse hosts in excess when placed with a high number of *L. trifolii* larvae.

20-233

INCIDENCE OF PATHOGENS AND PARASITOIDS OF *Anticarsia gemmatilis* (LEPIDOPTERA: NOCTUIDAE) PRE-IMAGINAL IN A SOYBEAN (*Glycine max*) AGROSYSTEM, RS, BRASIL.

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Information about damage potential to soybean by the soybean caterpillar (*Anticarsia gemmatilis*) is necessary before we can establish the control level. This study employed the life systems analysis of *A. gemmatilis* and their natural enemies. (pathogens and parasitoids). The pre-imaginal stages were collected twice a week in the agricultural year 1995/1996. Ten different plant samples were alleatoty collected and they were checked for the egg's presence in the leaveslets. The caterpillars were captured by the Sheppard's Method and analysed under laboratory conditions (14L:10D; 25°C; 70%RH). The results showed differents frequencies of parasitoids and pathogens in eggs, caterpillars and pupae related with fenological plant stage. The egg's parasitoid was only *Trichogramma pretiosum*. In the larval stages, we observed 81% mortality caused by diseases, 18% by parasitoids and 1% by others ones. The diseases more frequent were *Entomophthora* sp (40%) and *Baculovirus anticarsia* (21%) and the parasitoid observed was *Microcharops* sp (Hymenoptera). In the pupal period 89% of mortality detected was caused by Tachinidae. The statistical analysis showed correlation between the plant fenology, the frequency of the three principal pre-imaginal stages of Noctuidae and the incidence of their natural enemies.

20-234

HOW TO RECOVER BENEFICIAL PARASITOIDS OF THE ALFALFA SEED CHALCID, *BRUCHOPHAGUS RODDI* GUSS. (HYMENOPTERA: EURYTOMIDAE) FROM THE "GARBAGE" ?

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The seed-boring larvae of the alfalfa seed chalcid, *Bruchophagus roddi* Gussakovsky (Hymenoptera: Eurytomidae) can cause damage up to 80% in the production of alfalfa sowing-seed. The larvae of this pest host a number of beneficial parasitoids, which are in the stage of diapausing larva at the time of commercial seed-harvest. At commercial seed cleaning, seeds with a diapausing parasitoid larva inside go to the chaff, and they will be eliminated with the chaff. We suggest recycling beneficial parasitoids from this natural reservoir in order to use them as biocontrol agents against the alfalfa seed chalcid. A simple method to re-gain the beneficial parasitoids from the chaff, to store them until use, and to let them emerge is proposed. Possibilities to separate beneficial parasitoids from their non-parasitized hosts are discussed.

20-235

MATING STRUCTURE AND SEX RATIO IN A SEED EATER BRACONID WASP *ALLORHOGAS DISPISTUS* AND IN ITS PARASITOID *EURYTOMA* SP (EURYTOMIDAE)

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Larvae of *Allorhogas dispistus* feed on developing seeds of *Pithecellobium tortum* (Leguminosae) an abundant and widely distributed shrub in the coastal scrub (restinga) of Barra de Maricá, Maricá County, Rio de Janeiro State, Brasil. These larvae can be parasitized by the solitary parasitoid *Eurytoma* sp. In order to describe the mating structure and the sex ratio of both species, 551 fruits from 13 plants were collected from June to August 1995, and observed daily for adult emergence. In all 1751 braconids and 3991 eurytomids were obtained. In both species males tend to emerge earlier than females. *A. dispistus* aggregated in groups of up to eight males waiting for each emerging female showing no aggressive behavior. *Eurytoma* sp males also wait for emerging females but males are very aggressive and only one wins the contest. In both species only mated females disperse. These observations are consistent with the Local Mate Competition hypothesis (Hamilton, 1967; Science 156: 477-88) from which we would expect a female biased sex ratio. However, summing all insects that emerged throughout the whole season the real sex ratio is 1:1 both for the braconid and the eurytomid. This can be explained by the observed male dispersal from the natal patch, which would put this case into the intermediate mating structure category, especially in this case where patches are abundant and widely distributed.

20-236

HOST-PARASITOID RELATIONSHIP IN ENTOMOGENOUS GALLS IN *PAULLINIA CARPOPODEA* (SAPINDACEAE) IN THE SOUTHEAST BRAZIL

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Very little is known about the ecology of galls and their associated insects in the neotropical regions. In an Atlantic Forest ecosystem in the Southeast Brazil we found 11 different types of entomogenous galls in *Paullinia carpopodea*. Nothing has been recorded in the literature about these or any other galls from this plant species. Our objective was to describe the composition, dynamics and structure of these galls and their associated insects.

Twenty plant individuals were twice a month checked for galls. Some of these galls were collected for morphological analyses and to obtain the insects in the laboratory. Most of the galls were found throughout the year, possibly due to the constant production of new leaves, which was observed.

Ten types of galls are induced by Cecidomyiidae (Diptera) from which we obtained 28 wasp species belonging to 7 different families: Eulophidae (64%); Eurytomidae (18%); Mymaridae, Trichogrammatidae, Pteromalidae, Encyrtidae and Scelionidae (18% all together). The Eulophidae predominance in the gall parasitoid guild has been observed in other studies in this region, and it is quite different from data available for the nearctic region. If the parasitoid species represented by a single individual were excluded, it would be found that 70% (12 species) of them occur in only one type of gall, that is, they are probably specialists in this system.

From only one type of gall, there were 54% of the insect species. This high diversity can be explained by its high abundance and complexity caused by phytophagous agents acting as gall modifiers (endogall).

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20-237

MUSCA DOMESTICA L. 1758 AND ITS PARASITOIDS IN SOUTHEASTERN BRAZIL.

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A survey was conducted to verify sazonal abundance of *Musca domestica* L. and its parasitoids, in microhabitats of a dairy cattle farm. *Spalangia cameroni* Perkins, *S. endius* Walker, *S. nigroaenea* Curtis, *S. gemina* Boucek and two species of *Muscidifurax* (Hymenoptera: Pteromalidae) were found associated with *M. domestica*. Larvae of *Aleochara puberula* (Klug) (Coleoptera: Staphylinidae) also emerged from *M. domestica* puparia and these larvae were kept in conditions of laboratory to wait the emergence of adults. *A. puberula*, *S. cameroni* and *S. endius* were the most important species parasitizing *M. domestica*, although they showed different sazonality. The population of *M. domestica* was influenced by temperature and precipitation, but parasitoid species were influenced by the flies density, showing the possibility that could be a density-dependence relationship.

20-238

THE USE OF LIFE TABLES IN ASSESSING THE ROLE OF INSECT PREDATORS AND PARASITIDS AS MORTALITY FACTORS OF THE APPLE ERMINE MOTH, *YPONOMEUTA MALINELLUS* (LEPIDOPTERA: YPONOMEUTIDAE)

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The apple ermine moth (AEM), of European origin, was accidentally introduced into British Columbia and Washington State in 1981 and developed into a serious pest of apple trees. The larvae of *Yponomeuta malinellus* ZELLER feed in characteristic tents monophagous on apple leaf clusters and may defoliate entire trees during heavy infestations, thus causing economic losses.

In Europe, AEM are attacked by a large number of natural enemies. Life table studies including fertility estimates of AEM were carried out to understand the role of natural enemies in controlling the pest in Europe. From the parasitoid community of *Y. malinellus* in Germany 7 different primary parasitoid species were obtained, which belong to 4 different parasitoid guilds attacking distinct niches in the life cycle of AEM. Life tables indicate that insect egg predators in the present study were abundant and that the impact of these predators reached 34.9% in 1993, 37.0% in 1994, and 50.8% in 1995 of the total generational mortality of *Y. malinellus*. The impact of the parasitoids in relation to the total generational mortality of *Y. malinellus* was 19.0% in 1993, 13.1% in 1994, and 14.4% in 1995. Life tables also indicated that the population dynamics of the AEM is significantly influenced by the failure or attainment of AEM females to realise their potential fertility.

20-240

EVALUATION OF TWO PARASITIDS (HYM: BRACONIDAE) FOR THE CONTROL OF *APHIS GOSSYPYII* GLOV. AND *MYZUS PERSICAE* SULZ. (HOM.: APHIDIDAE) ON PEPPER

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Some trials were conducted on a pepper protected crop in order to evaluate the efficacy of *Aphidius colemani* and *Lysiphlebus testaceipes* as biological control agents on *Aphis gossypii* and *Myzus persicae* populations.

These parasitoids appear frequently in this crop and showed different host preferences and parasitization rates.

Aphidius colemani showed no preference between *Aphis gossypii* and *Myzus persicae*, and a good control on both aphids was observed.

Lysiphlebus testaceipes showed a preference only for *Myzus persicae*.

20-239

GEOGRAPHIC DISTRIBUTION OF PARASITIDS OF THE CARIBBEAN FRUIT FLY, *ANASTREPHA SUSPENS*A, IN FLORIDA
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A survey was performed to determine the geographic distribution of braconid larval-pupal parasitoids of the Caribbean fruit fly. Host fruit (loquat, Surinam cherry, Cattley guava and common guava) were collected throughout central and south Florida. Fly larvae pupated in moist vermiculite, and emerging parasitoids were identified.

Diachasmimorpha longicaudata was limited to south Florida, reaching higher latitudes along the Atlantic coast. *Doryctobracon areolatus* was the dominant species in the majority of interior locations, its numbers decreasing toward the northern limit of host distribution. It was uncommon along the western coast of the peninsula, and absent along the eastern coast and the southern edge of Lake Okeechobee. Its absence on the southeast coast, where it was originally released and established, suggests that a process of displacement has occurred. The ranges of *D. longicaudata* and *D. areolatus* overlap only within a limited area, and only at one interior location were both common. *Uteetes anastrephae* was widespread, with the largest numbers being recovered from the southeast and southwest coasts, and along the southern edge of Lake Okeechobee. The different distribution patterns of the three species stress the importance of selecting biocontrol agents that are adapted to the environmental conditions of the target release area.

20-241

INTERACTIONS BETWEEN A TACHINID PARASITOID AND A GRANULOSIS VIRUS OF THE WESTERN GRAPELEAF SKELETONIZER

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Interactions between the tachinid parasitoid, *Ametadoria misella*, and the granulosis virus (GV) of the western grapeleaf skeletonizer, *Harrisina brillians*, were investigated.

In a field study, the ovipositional preference of *A. misella* for healthy or HbGV infected *H. brillians* at 3rd or 4th instar was studied to investigate the degree of interspecific competition between the natural enemies. Treatments were significantly different as determined by ANOVA ($F_{2,6}=5.97$, $p=0.04$). Comparison of individual means by pooled t-test using Bonferroni's adjustment revealed that *A. misella* oviposited, as determined by dissection, 6 times more often ($t_4=5.98$, $p<0.01$) in 4th instar *H. brillians* larvae (mean= $51\%\pm 10\%$ SD) than in 3rd instar hosts ($8\%\pm 7\%$); and 3 times more often ($t_4=2.235$, $p=0.08$) in healthy *H. brillians* larvae ($51\%\pm 10\%$) than in symptomatic HbGV infected larvae ($16\%\pm 25\%$). Additionally, a lower proportion ($F_{1,2}=5.53$, $p=0.14$), although not significantly different, of field collected *H. brillians* pupae parasitized by *A. misella* tested positive for HbGV by ELISA than nonparasitized pupae; and the mean concentration of virus, estimated by ELISA absorbance₄₀₅, in parasitized *H. brillians* pupae (0.76 ± 0.39) was significantly lower ($t_{64}=3.616$, $p<0.01$) than in nonparasitized pupae (1.27 ± 0.45). These results suggest a mechanism of stage-specific host partitioning by natural enemies which reduces interspecific competition for hosts.

The potential of *A. misella* to transmit HbGV to healthy *H. brillians* after emerging from virus infected hosts was investigated. HbGV was not detected by ELISA or bioassay in whole insect or meconium homogenates of *A. misella* emerging from HbGV-infected *H. brillians*.

20-242

ENZYMATIC ANALYSIS OF *APANTELES GLOMERATUS* (HYMENOPTERA: BRACONIDAE)

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Apanteles glomeratus (Hymenoptera: Braconidae) is a parasitoid of *Pieris brassicae* (Lepidoptera: Pieridae) larvae in Azores Islands. This specie was characterised biochemically to improve the systematic basis of this genus. Seven enzyme systems studied by electrophoresis were analysed as aldehyde oxidase (AO), α -glycerophosphate dehydrogenase (α -GPD), tetrazolium oxidase (TO), malate dehydrogenase (MDH), glucose-6-dehydrogenase (G6PD), malic inzyme (ME), isocitrate dehydrogenase (IDH). All systems present only one band, with two exception: the AO presented two bands and the α -GPD presented three bands which corresponded to different locus. No polymorphic bands were detected.

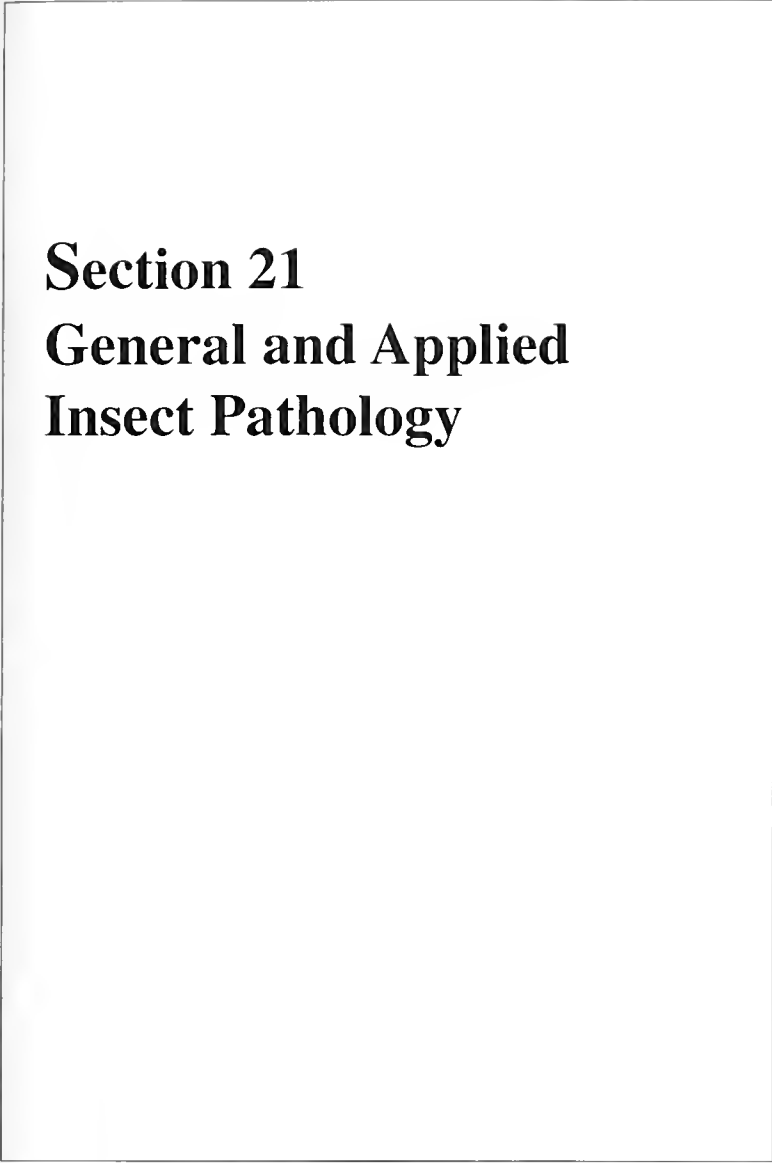
20-243

THE TRENDS OF DIAMONDBACK MOTH PARASITIDS ON CABAGGE IN RELATION TO DIFFERENT MIXED CROPS

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Diamondback moth (DBM), *Plutella xylostella* (L.) Is the most important pest on cabbage in North Vietnam. The population fluctuations and abundance of the pest were investigated weekly on cabbage (*Brassica oleracea* L. . *B. gongilodes* L.) from November to March of two years 1992-1994. The investigations carried out in two localities of Red River Delta showed that of two important parasitoids reared from DBM, the pest pupae parasitized by the pupal parasitoid *Phaeogenes* sp. (Ichneumonidae) was very low (maximum 3.3%) in comparison with the sole larval parasitoid *Cotesia* (= *Apanteles*) *plutellae* Kurdj. (Braconidae) (average 30.8%).

It was found that cabbage fields adjacent to maize and rice fields often have significantly lower parasitized pest population by *C. plutellae* than those on cabbage intermixed with different crops of Solanaceae, Fabaceae groups and some other decorative flowers (Asteraceae), for the maximal proportion of parasitized pest larvae was less than 25% (average 13.1%) on cabbage grown alone compared to percentage of parasitism up to 40% (average 17.7%) on the mixed planting cabbage with different crops.

In addition, cabbage fields of both above localities often treated by some pesticides as Cartap (PADAN 95BHN), Phethoate+Enthofenprox (CIDI M) but it was observed that the peak of parasitoid populations usually occurred in the middle and towards the end of the crop.



Section 21

General and Applied

Insect Pathology

21-002

OCCURRENCE AND USE OF ENTOMOPATHOGENIC NEMATODES IN NEW ZEALAND

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New Zealand has a varied nematode fauna consisting of both exotic and endemic species. The entomopathogenic rhabditids are well represented by steinernematid and heterorhabditid nematodes examples of which have been isolated from soil samples and insects. These have been characterised by morphometrics and genetic analysis (PCR/RAPD). Strains of *Steinernema feltiae* have been frequently isolated from native and semi-improved hill country grasslands, suggesting that strains of this species are endemic. *Steinernema carpocapsae* and *S. glaseri* have also been isolated, but are less common and could be of exotic origin as both species have been released into the country during biological control experiments. Heterorhabditid nematodes corresponding to *H. bacteriophora* and *H. zealandica* have also been isolated, most frequently from coastal areas. Entomopathogenic nematodes, however, are seldom isolated from improved pastures, where insect pests abound, although laboratory and field tests show their potential as control agents. Heterorhabditid nematodes have been effective against pasture scarab pests and a New Zealand strain of *S. feltiae*, showing low temperature activity, has been tested and found effective against soil dwelling hepialid larvae in winter. High production costs of entomopathogenic nematodes currently preclude their use as inundative control agents in broad scale agriculture. Heterorhabditid nematodes, however, have been registered and are used commercially for control of weevil pests in plant nurseries.

21-001

HETERORHABDITIS MEGIDIS POINAR ET AL.;
A BIOLOGICAL CONTROL AGENT OF FOREST INSECTS AND A CARRIER OF ANTIFUNGAL AGENTS.
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Heterorhabditis spp. and their symbiotic bacteria, *Photorhabdus luminescens* (Thomas and Poinar) Boemare et al., have been shown to be virulent against certain forest coleoptera, diptera, lepidoptera and hymenoptera, and to have potent fungicidal capabilities. In laboratory experiments *H.megidis* Poinar et al., caused a variable mortality among forest insects, namely 27% in *Contarinia oregonensis* (Foote), 56% in *Strobilomyia appalachensis* Michelsen, 100% in *Choristoneura fumiferana* Clemens, 100% in *Diprion similis* (Hartig) and 30% in *Otiorhynchus ovatus* L. The antibiotic, 3, 5 - dihydroxy - 4 - isopropylstilbene produced by *P.luminescens* has strong fungicidal activity with minimum inhibitory concentrations ranging from 12 µg/ml against *Cryptococcus neoformans* and *Botrytis cinerea* to 25µg/ml against *Aspergillus flavus*. Exo-chitinase and endo-chitinase also is produced by *P.luminescens* and, as well as being fungicidal, they may help the nematodes to exit the insect cadavre by softening the insect cuticle.

21-003

THE ROLE OF MERMITHID NEMATODES IN THE OUTBREAK DYNAMICS OF WINGLESS GRASSHOPPER, *PHAULACRIDUM VITTATUM* (SJÖSTEDT), IN AUSTRALIA

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In the tablelands of south eastern Australia, small scale, chronic infestations of wingless grasshopper persist throughout the recessionary phase of the outbreak cycle in habitats unfavourable for mermithid nematodes (hot-spots), typically steep terrain with low soil moisture. Ironically, reliable host availability at hot-spots ensures the persistence of a reserve, albeit at a low level, of mermithid nematodes during the recessionary phase. The population surplus produced at hot-spots seasonally disperses to adjacent moist habitats (primary invasion areas) where, under average seasonal rainfall conditions, they are decimated by abundant mermithid nematode populations. Under drought conditions there is curtailment of activity by mermithid nematodes which results in (a) high host survival in hot-spots (b) an exceptionally large host population surplus which invades and breeds successfully in primary invasion areas and (c) the production of a high proportion of long-winged morphs at hot-spots, a consequence of high density, which disperse widely to infest grasslands made favourable for habitation by the drought (secondary invasion areas). The lack of biological control agents in secondary invasion areas results in unrestrained increase in wingless grasshopper populations relatively independent of seasonal rainfall conditions. This population growth consolidates infestations giving rise to regional outbreaks. Outbreak collapse occurs sequentially in the different habitats. In hot-spots and primary invasion areas collapse follows immediately after the return of average seasonal rainfall and consequent renewal of nematode activity. In the more extensive secondary invasion areas there is a delay of 1-2 seasons during which nematode populations establish and increase to ultimately cause localised host extinction.

21-004

HIGH TEMPERATURE STEINERNEMATIDS: CAN THEY BE USED IN PEST MANAGEMENT

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A new species of *Steinernema* has been recovered from soil in an alfalfa field in the Sultanate of Oman where bollworms, particularly *Helicoverpa armigera*, are the main insect pests. The new species is taxonomically distinct from *S. riobravus* which was recovered from prepupae and pupae of *H. zea* and *Spodoptera frugiperda* in Texas, USA. The thermal niche breadth for infection and establishment of the two species is similar and both species will reproduce in *Galleria mellonella* between 25 and 25°C. The LT₅₀ and LC₅₀ of the two species are very similar.

Various bollworm species are the principal targets for these "high temperature" steinernematids. The paper discusses the problems of introducing new species of entomopathogenic nematode into pest management programmes.

21-006

RESEARCH ON ENTOMOPATHOGENIC *STEINERNEMA* AND *HETERORHABDITIS* IN CHINA

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Steinernema and *Heterorhabditis* nematodes have been widely used for the control of insect pests as biocontrol agents in soil, cryptic and aquatic habitats, and on foliage because of their wide range of insect hosts, their ability to search for and kill hosts rapidly, ease of mass rearing and environmental safety.

In China, since the 1950's, especially the late 1970's, various species or strains of *Steinernema* and *Heterorhabditis* have been introduced from Czechoslovakia, Australia, the United States and other parts of the world, and isolated from soil or/and natural insect hosts. The current status of entomopathogenic nematode research in China was summarized as follows.

Collection and identification of *Steinernema* and *Heterorhabditis*

- * Major isolates of *Steinernema* and *Heterorhabditis* in China
- * Identification

Application of *Steinernema* and *Heterorhabditis* for pest control

- * Assays on host range
- * Field development
- * Impact on non-target organisms

Symbiotic *Xenorhabdus* and *Photorhabdus* bacteria

- * Isolation and identification of *Xenorhabdus* and *Photorhabdus*
- * Recombinations of *Photorhabdus* and *Heterorhabditis* strains

Mass production of *Steinernema* and *Heterorhabditis*

- * Improved medium ingredients
- * Optimization of the culture parameters for nematode production

21-005

EFFECT OF AGE AND NEUTRAL LIPID CONTENTS ON MOBILITY AND INFECTIVITY OF *STEINERNEMA FELTIAE*

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A Finnish (SFS-22) and a Swedish (Umeå) isolate of *Steinernema feltiae* were stored for 1040 and 970 days, respectively, in moist polymer gel at 20°C and bioassayed at 1-3 months' intervals to study the effect of age and lipid contents on mobility and infectivity in a mobility test where infectives had to travel a 9 cm distance in sand cylinders to reach bait larvae (*Tenebrio molitor*), and in an infectivity test where nematodes were placed in the close vicinity of bait larvae in 5 ml sand pots. Some SFS-22 batches were transferred to 25°C after a year's storage at 20°C to obtain nematodes of similar physiological but of different chronological age. Lipid contents of infectives were measured with image analyzer after staining lipids with Oil-Red-O.

Due to clean storage conditions mortality of both isolates reached only 55-60 % by the end of the experiment. Both isolates were infective till the end, despite that about 90 % of infectives were devoid of lipids. Mobility decreased more quickly than infectivity. In sand cylinders, the mortality of baits and number of nematodes reaching the bait larvae dropped to a low level after the proportion of infectives devoid of lipids began to increase but in the infectivity test these parameters remained on the original level for 900 days. In time, infectivity and mobility fluctuated in close concordance with each other for SFS-22, but seemed not to be as tightly linked for the Umeå isolate. There were distinct differences in the pattern of activity of the two isolates in relation to time and depletion of lipid resources. Storage temperature affected the performance of aging nematodes as the mobility and infectivity of SFS-22 from 25 °C was always less than infectivity of SFS-22 from 20°C with similar lipid contents but of greater chronological age.

21-007

SALINITY EFFECTS ON THE INFECTIVITY AND HEAT TOLERANCE OF THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS* SPP.

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Entomopathogenic nematodes of the genus *Heterorhabditis* are frequently found in sandy coastal soils, and it has been suggested that the genus evolved from marine ancestors. We compared the performance of *Heterorhabditis* infective juveniles in sea water and distilled water. Isolates of each of the three taxonomic types found in Europe were tested: *H. bacteriophora*, and *Heterorhabditis* sp. of the North-West European and Irish Groups. Sea water protected the infective juveniles against the lethal effects of high temperatures, but the infectivity of all types for *Galleria mellonella* larvae was reduced in sand moistened with sea-water compared to that in sand moistened with distilled water. The basis for these effects, and differences between types, will be discussed.

21-008

SOIL - INHABITING PESTS AS THE HOSTS FOR THE ENTOMOPATHOGENIC NEMATODES

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Entomopathogenic nematodes in the families *Steinernematidae* and *Heterorhabditidae* are widely regarded as being excellent biological control agents for a number of insect pests in soil. The research on natural enemies of the apple sawfly - *Hoplocampa testudinea* Klug., a serious pest of sub - carpathian orchards, undertaken 20 years ago enable to isolate, for the first time in Poland, a newly described genus *Heterorhabditis*. Semi - field trials showed a high effectiveness of the nematodes, applied into soil, against the apple sawfly. In micro-plot experiments nematodes applied in high doses prophylactically through watering young seedlings of cauliflower, onion and carrot plants, protected them from the relevant fly pests as efficiently as chemical treatments. No effect of entomopathogenic nematodes on the mortality of *Ichneumonidae* inside cocoons of apple sawfly larvae or closed in *Delia* pupariums was found.

The recent studies revealed that other groups of soil - inhabiting pests were also suppressed by entomopathogenic nematodes, thus biological pest control on vegetable crops could be extended into other agrophagous invertebrates such as terrestrial isopods (*Porcelio scaber* Latr.) millipedes (*Blaniulus guttulatus*) and even slugs (*Deroceras*, *Arion*). However infective juvenile production of nematodes was not completed in every case.

21-010

SPECIES IDENTIFICATION OF THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS* BY MOLECULAR AND CROSS-BREEDING TECHNIQUES

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Heterorhabditis is an important agent for the biological control of insect pests in the soil. The complex life-cycle of *Heterorhabditis* nematodes makes them less amenable for introducing beneficial traits by means of interstrain crosses, which would make them more effective in field application. First generation *Heterorhabditis* hermaphrodite females give rise to a second generation which contains amphimictic females and males and hermaphrodite females. The nutrient status of the environment in which larval development occur plays an important role in determining the ratio of these progeny types.

However in carefully controlled experiments, the amphimictic females can be selected and used for genetical crosses. By these crosses new traits could be introduced and also biological species could be identified among the large number of isolates collected in recent years.

Details of these crosses and comparison of species identification by crosses vs. PCR amplification will be presented. Our present understanding of the life-cycle, which is important in utilising the genetic approach to strain improvement, will also be discussed.

21-009

GENETIC IMPROVEMENT OF HEAT TOLERANCE IN *HETERORHABDITIS BACTERIOPHORA* POINAR.

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Exposure to environmental extremes such as high temperatures may hinder the biological control efficacy of entomopathogenic nematodes. Therefore research has been initiated to find or develop superior nematodes through genetic improvement. In the present study, a heat tolerance trait was transferred from one strain of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar to another. The trait was transferred from the heat tolerant IS5 strain to the commercial HP88 strain through controlled crosses. The hybrid nature of the progeny was verified using a marker mutant of the HP88 strain (*Hp-dpy-2*). Heat tolerance in the hybrid progeny was similar to the IS5 strain and significantly greater than the HP88 strain. The virulence at high temperatures (32°C) of the IS5 and hybrid nematodes was greater than HP88 nematodes. Similar to the IS5 strain, the hybrids displayed sensitivity to cold. No differences were detected in reproductive potential among the strains. This study demonstrates the promise of using hybridization to genetically improve entomopathogenic nematodes, and illustrates the advantage of using marker mutations in this endeavor.

21-011

STUDIES ON THE GENUS *HETERORHABDITIS* POINAR, 1975 IN ARGENTINA.

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The genus *Heterorhabditis* appears as the most extensive distribution in Argentina. It was recovered in a large diversity of milieu including near Atlantic and Andes mountains areas and the central region of the country. It was frequently founded in cultivated and natural areas. The work carried out in the Laboratorio de Nematología, Centro de Zoología Aplicada, comprised several subjects.

A) Survey and detection: soil and insects samples arising from different localities of the country were analyzed. At present all the isolates belong to the species *H. bacteriophora* Poinar, 1975. This shows the plasticity and variability that characterizes the species.

B) Isolates characterization: it is realized on the basis of the analysis of several characters. 1) Morphologiques. Taking into account the stages commonly considered no significant differences were observed among isolates. 2) Morfométriques. The greatest variability was observed in hermaphrodites and in females; the minor in males and infective larvae. According to the stage the degree of variability is different. That variability appears closely associated with the conditions of milieu (environment and host). 3) Life cycle. It was observed a succession of three hermaphrodite generations and an only one amphimictic, this one was overlapped to the second hermaphrodite. The disponibility in food influences the modality and the time parasitaire. Generally, the bigger the infection, the shorter the cycle and the faster the production of new infectives. 4) Patterns Distribution in soil. Horizontal and vertical patterns in infective larvae were aggregative. Concerning the layer preference the larvae were often found between 10-30 cm. in depth. 5) Specificity and Infectivity. Specificity appears mainly conditioned by ecological factors. Isolates showed different aggressive degrees in relation to determined host.

Up to now, the data contribute to the knowledge of *H. bacteriophora* and they are orientated to the selection of useful isolates for the control against harmful insects.

21-012

ISSUES AND CONCERNS REGARDING ENTOMOGENOUS NEMATODES

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The field of entomogenous nematodes has developed rapidly during the past two decades. The great majority of this activity has been due to studies on the entomopathogenic nematodes *Steinernema* and *Heterorhabditis*. Aside from the many advances made in this complex area, there is now a need to address the following basic concerns.

1. What type of information should be used for the basis of a species concept: morphological, genetic or molecular?
2. If morphological, which characters are the most stable in light of geographical variation? If molecular, which sequences on which genes will be the most useful for species differentiation and which should be used to detect intra-specific variation?
3. How important is species and strain diversity in relation to host selection?
4. What are the geographical ranges of species of *Steinernema* and *Heterorhabditis*? How many of these species are inter-continental? Are some described species simply strains of other described species? How rapidly does speciation occur in these genera?
5. What are the natural means of distribution of species in these genera and how does this relate to speciation? How important are abiotic factors in the development of new species?

Regarding entomogenic nematodes in general, the fossil record can tell us about the past distributions of nematodes and their hosts, the antiquity of insect parasitism by nematodes and explain present day host preference phenomena.

21-014

THE INDIRECT EFFECT OF PLANT HOST ON THE SUSCEPTIBILITY OF SITONA LINEATUS L. TO ENTOMOPATHOGENIC NEMATODES

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The pea and bean weevil *Sitona lineatus* (Col., Curculionidae) is an important pest of leguminous crops in many parts of the world. In Poland, the pest status of *S. lineatus* has increased within the last years. Adult weevils overwinter in perennial legumes such as lucerne and clovers. After migration to annual legume crops in spring, weevils mate and lay eggs. Larvae feed on the nitrogen fixing root nodules. Previous attempts using entomopathogenic nematodes (EN) for microbial control of this insect have been directed against young instars from clover stands and showed that these weevils are susceptible to EN. Field trials showed that *S. lineatus* were most abundant in early spring period on plots with various cultivars of pea. In May in egg laying period, bean cultivars became more attractive. Laboratory studies on the preference for annual legumes by pea weevils showed that activity and natural mortality of *S. lineatus* adults may be connected with host plant preference which influences feeding activity, average body weight and susceptibility to EN. Larvae of *S. lineatus* were shown to be the most susceptible stage for infection by EN, especially those feeding on peas. The nematodes were able to reproduce effectively in all cadavers, but juveniles of EN emerged in greater quantities from *S. lineatus* larvae from beans than with those from peas. The greatest number of infective juveniles was obtained from *S. lineatus* larvae from late field bean. Adults of the new summer generation of *S. lineatus* from pods reached higher mortality rates than those from beans. These results indicate the importance of including different host plants in testing nematodes against insect species.

21-013

UTILIZATION OF *CULICOIDES VARIIPENNIS* LARVAE BY THE MERMITHID NEMATODE *HELEIDOMERMIS MAGNAPAPULA*, AND INTERACTIONS WITH IRIDESCENT VIRUS INFECTION

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Aquatic larvae of the blood-feeding midge and bluetongue virus vector *Culicoides variipennis sonorensis* are parasitized by a mermithid nematode, *Heleidomermis magnapapula*. Young host larvae (L1 & L2) are preferred for parasitism in the laboratory and field, leading to successful development and emergence from late instar larvae (L4). Distribution of adult nematodes in the field allows optimal contact with young hosts. In the laboratory, preparasites take longer to enter older hosts, and older larvae are more likely to encapsulate parasites or to eat the preparasites before they have an opportunity to penetrate. Still, parasitism of late instars probably is critical for nematode dispersal among spatially or temporally isolated developmental sites (e.g. dairy wastewater ponds) via parasitized adult midges.

Host larvae in one dairy wastewater pond were concurrently infected with iridescent virus; virus infection reached 30% or more among second instar hosts. Infected larvae in the field were essentially all parasitized by *H. magnapapula*. Exposure of naive host larvae to virus in the laboratory resulted in <2% infection, while exposure in the presence of mermithid preparasites caused nearly 100% virus infection, probably due to virus introduction into the host hemocoel by the nematode. Virus incubation (4-5 days) is less than time required for successful parasite maturation (12-15 days), leading to death of both mermithid and host. This results in an interesting scenario, in which the superior biological control agent, *H. magnapapula*, suffers, while an inefficient pathogen benefits. The evolutionary implications of this relationship will be discussed.

21-015

THE AREA OF DISCOVERY AND HOST SEARCHING STRATEGY OF AN ENTOMOPHAGOUS NEMATODE *HETERORHABDITIS BACTERIOPHORA* IN SOIL COLUMNS.

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The host searching behaviour of infective juveniles (IJs) of *Heterorhabditis bacteriophora* was ascertained in terms of their area of discovery and vertical migration in the soil columns in a laboratory bioassay. In assessing the potential of *H. bacteriophora* as a promising biological control agent, it is a prerequisite to understand the searching capacity of the IJs in their microniche i.e. soil. The area of discovery of IJs of *H. bacteriophora* was observed to be affected by the density of the host larvae in the soil column and it is calculated to be 0.176. Vertically the IJs migrated down ca. 15cm in the soil-column and were able to parasitize successfully 66.66% of the *Spodoptera litura* (Lepidoptera: Noctuidae) larvae, the target host, within two days interval. The searching efficiency with respect to various other parameters is also discussed.

21-016

Foliage Protection And Mortality Reduction In Prestressed Hardwood Forests During A Massive Outbreak Of Gypsy Moth (*Lymantria Dispar* L.)

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From 1992 - 1995 the deciduous hardwood forests in the plains and hillbelts of Germany were confronted with the heaviest outbreak of Gypsy Moth in the recent forest history. In 1994, the year of culmination, a total amount of 190000 acres was infested.

In Hesse, the aerial counter treatments were used to perform an experimental programme to optimize the foliage protection and to monitor reactions of unprotected stands.

First results of these long-term field trials are:

1. The pattern of Gypsy Moth distribution was disjunctive, often due to wind drift among first and second instar larvae.
The egg masses were concentrated in heights of more than 4 meters above ground. In areas with high population densities the ground vegetation had been totally destroyed subsequent to crown defoliation. Only *Prunus serotina*, *Impatiens noli-tangere*, *Deschampsia flexuosa* and *Urtica dioica* were resistant.
2. Up to an egg mass density of 10 per tree *Bacillus thuringiensis* (Btk) - applications with FORAY 48B^R (4l/ha; 50,4 x 10⁹ IU) and DIPEL ES^R (2l/ha; 33,4 x 10⁹ IU) showed good efficacy compared to the one of DIMILIN^R (Diflubenzuron 150 g/ha).
The stand density and vertical structure had an important influence: stands with two or more layers showed minor foliage protection after Btk-applications.
3. Dimilin^R - treated areas at first sight revealed more negative ecological effects than Btk-areas. Long-lasting effects cannot be proved yet.
4. Stands with intact ground water regime showed no remarkable damage.
Total defoliation in predisposed stands reduced the radial growth of trees down to zero.
Only those trees which had been protected reached their prior growth rates.
5. The current monitoring programme will reveal further results for the management of valuable mixed natural stands in the Rhine-Main-area.

21-018

INCESTICIDAL ACTIVITY OF *BACILLUS THURINGIENSIS* SUBSP. *KURSTAKI* HD-1 AND SUBSP. *SOTTO* BIOTYPE *DENDROLIMUS* AGAINST *DENDROLIMUS PINI* (L.) (LEPIDOPTERA: LASIOCAMPIDAE)

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The pine moth (*Dendrolimus pini* L.), a serious primary pest of Scots pine (*Pinus sylvestris* L.) in Poland, was controlled over a total area of about 200 thous. ha in 1950-95.

The increase of *B. thuringiensis* (B.t.) spraying range stimulates to a searching of B.t. strains more efficient in pests control. The insecticidal activity of B.t. subsp. *kurstaki* HD-1 and B.t. subsp. *sotto* biotype *dendrolimus* against 2nd and 5th instars larvae of the pine moth was compared. Bioassayed larvae were infected during a feeding on pine needles dipped in B.t. suspensions. Mortality of larvae reared at 25°C, 70-75 % RH and D:L - 16:8 was checked and LT₅₀ was calculated. For 5th instar larvae the number of frass was counted. Experiments showed that B.t. subsp. *sotto* biotype *dendrolimus* seems to be more promising in the control of *D. pini* than B.t. subsp. *kurstaki* HD-1.

21-017

MICROBIAL CONTROL OF GYPSY MOTH DURING THE GRADATION 1992 - 1994 IN GERMANY: RESULTS OF FIELD TRIALS USING *BACILLUS THURINGIENSIS* AND NUCLEAR POLYHEDROSIS VIRUS

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The last mass outbreak of gypsy moth in Hesse, Germany, was used to compare different concentrations and also mixtures of *Bacillus thuringiensis* (B.t.) and Nuclear Polyhedrosis Virus (NPV). The results of these field trials, using aerial and ground application, can be summarized as follows:

- 1) Application rates of B.t., as approved by the registration authorities and used by the forest service up to 1994 (1.5·10¹⁰ IU/ha), were too low to get adequate control of gypsy moth.
- 2) Since 1994, the registration of B.t. preparations covers application rates similar to the rates used in North America (1·10¹¹ IU/ha). Use of these new, higher rates lead to excellent control of first instar larvae of gypsy moth and good protection against defoliation.
- 3) Egg mass treatment from the ground with NPV resulted in a high virus infestation of the gypsy moth population, but was not able to prevent defoliation.
- 4) Two treatments with a high concentration of NPV (1.3·10¹² Polyhedra / ha) gave an acceptable level of protection against defoliation and an excellent reduction of the gypsy moth population.
- 5) Combining B.t. and NPV was particularly promising (and economically attractive) when both pathogens were applied at relatively low dosage. A mixture of 1/10 of the normal concentration of B.t. and NPV gave similar larval reduction and foliage protection as the full B.t. dose. The virus was established in the population and, thus, could give long term control.

In the following year (1995), at most locations, no further field trials were possible as the gypsy moth populations collapsed completely due to parasitoids and/or virus.

21-019

INFLUENCE OF EATING BY *THAUMETOPOEA PITYOCAMPA* SCHIFF. (LEPIDOPTERA: THAUMETOPOEIDAE) IN THE PROCESS OF THE GROWTH OF *PINUS BRUTIA* TEN.

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The main reasons for this experiment were the relatively large area the forests of *Pinus brutia* Ten. which exists in Greece, and the intensive attack from the needle-eating insect *Thaumetopoea pityocampa* Schiff. The aim of this treatment was the investigation of the effects that three successive total defoliations had on the growth of young trees. The field experiment was established in the estate of the Forest Research Institute and included:

- a) Total defoliations during the follow periods:
- 1986-87, 1987-88, 1988-89 (attack 86)
- 1987-88, 1988-89, 1989-90 (attack 87), and
- b) Measurements of height and diameter of the trees at every two months.

The results are the following:

- 1) No deaths (partially or totally) were observed, either to the control (full protection against any defoliation), or even to the total defoliation regimes applied.
- 2) Heights and perimeters of the defoliating trees were lower than those of the control (49,1-59,5%).
- 3) For two years after the end of the last defoliation the trees grew still slower than those at the control.

21-020

MICROBIAL CONTROL OF THE PINE PROCESSIONARY CATERPILLAR, *THAUMETOPOEA PITYOCAMPA* DEN. & SCHIFF., IN ITALY (LEPIDOPTERA: THAUMETOPOEIDAE)

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The Pine Processionary Caterpillar *Thaumetopoea pityocampa* is the most important defoliator of both native and introduced pines all over Italy. In particular it is the key species in young plantations and on ornamental trees. The life cycle is characterized by two striking features, as the gregariousness of the larvae and the prolonged diapause as a pupa in the soil, resulting in a high degree of adaptability to various environmental and climatic conditions.

A number of microbial antagonists have been isolated from larvae and pupae in the last 30 years. Some of them have been also used in experiments of biological control, providing encouraging results. However, only *Bacillus thuringiensis* has passed the experimental screening and it is now available for routine treatments, even if its application from the air is subjected to a strict limitation by national rules.

The high variability in effectiveness observed in the field after a single application of *B.t.* (20-85% of mortality) is related to a great variability in some population parameters of *Th. pityocampa*. The emergence period of adults varies from July (Alps) to October (South) and its duration is related to the spreading of the cohorts (individuals with life cycle of different length) over time. In addition, the embryonic development takes from 20 to 45 days, depending strictly on the temperatures recorded in the egg masses. As a result, the most vulnerable larval instars (L1 and L2) are never thoroughly reached by one application only, as the persistence of the bacteria on the needles is usually limited. Two applications are requested to control the pest in stands where the egg hatching occurs over more than one month.

21-022

THE FUTURE OF *BACILLUS THURINGIENSIS* IN FORESTRY.

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There are four commercially available endotoxin producing strains of *Bacillus thuringiensis* (Bt). Bt sps *kurstaki*, Bt sps *aizawai*, Bt sps *tenebriones*, and Bt sps *israelensis*.

The most widely used strain is Bt *kurstaki* (Btk.), which is employed for the control of forest defoliating lepidopteran larvae. Over 90% of North American forests that are treated for lepidopteran defoliators now employ Btk. Whereas in Europe only 30% of the treatment are now made with it, offering a considerable growth potential, as do other parts of the world whose forests, similarly affected by lepidopteran larvae. Bt *tenebriones* (Btt), shows promise for control of many defoliating chrysomelidae. Good results being obtained from a wide number of species. Interestingly, adult beetles from other defoliating coleopteran families have also succumbed after feeding on treated leaves.

Of the other two subspecies, Bt *aizawai* (Bta) could perhaps be used to treat lepidopteran larvae, if its economics were more favourable. Bt *israelensis* (Bti), on the other hand has yet to find a target.

21-021

USE OF *BACILLUS THURINGIENSIS* AND *BACULOVIRUS* FOR THE NUN MOTH *LYMANTRIA MONACHA* (LEPIDOPTERA: LYMANTRIDAE) CONTROL

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The nun moth is an insect occurring in outbreaks in coniferous stands in Europe. Due to the high population density of pests and the risk of a complete damage to stands, control treatments are to be carried out every several years.

Laboratory studies on the use of pathogenic microorganisms as bioregulators of the nun moth numbers showed that the larvae are sensitive to *Bacillus thuringiensis* and nuclear polyhedrosis virus.

The results of control treatments with different *B.t.* commercial products varied over years. At present ULV formulations used at the doses of 3-4 l/ha are efficiently applied in forest protection in Poland. In 1994 Foray 48B was used in an area of 150 thousand ha causing high mortality and collapse of the nun moth outbreak.

Disease of the nun moth larvae caused by LmMNPV was one of bioregulators of the pest in Europe in the past when the nun moth attacked mainly the Norway spruce stands. During the last two outbreaks (1978-1984 and 1992-1994) in Poland the nun moth occurred generally on the Scots pine and no viral epizooties were observed. The nun moth control treatments at pine stands with the polyhedra of LmMNPV showed a low infectivity of the virus which means that it can not be considered as a pathogen useful in the nun moth control programmes at Scots pine stands.

21-023

FLUORESCENT WHITENING AGENTS AS SUNLIGHT PROTECTANTS AND ACTIVITY ENHANCERS OF INSECT PATHOGENIC VIRUSES

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Fluorescent whitening agents (FWA's) are commonly used in detergents and in paper manufacturing to enhance whiteness in clothes and paper. These chemicals have also been shown to be excellent sunlight protectants for viruses, nematodes, and fungi. In addition, they act as viral activity "enhancers" in several host-virus systems. The discovery, effects, and properties of these chemicals will be discussed in terms of both virulence and host spectrum. In addition, the stability of the FWA's and relationship between structure and function will be discussed.

21-024

RECENT ADVANCES IN FORMULATING BT AND OTHER MICROBIALS FOR INSECT CONTROL

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Formulation of *Bacillus thuringiensis* (Bt), viruses, fungi, and nematodes each present interesting and unusual challenges. Some of these agents are not palatable to insects and sunlight and rainfall contribute to the loss of insecticidal activity in different ways. Formulation may alleviate these problems. Matrix-forming materials derived from cornstarch, corn flour, wheat gluten, casein or lignin extend the residual activity of Bt significantly better than conventional formulations. These materials can be used in granular or sprayable formulations and are easily modified to fit the needs of a particular pest-crop situation. Granular formulations of Bt have been extensively tested against the European corn borer in field corn. In rainy weather, Bt formulated in cornstarch stays longer in the whorl of the plant than Bt formulated conventionally. Laboratory tests demonstrated that neonate corn borers preferred to feed on granules made from pregelatinized corn flour even in the presence of fresh plant material. Sprayable formulations have been tested on cabbage against diamondback moth infestations and residual activity measurements have been conducted against cabbage looper larvae. In both cases, matrix forming materials protected Bt from both rainfall and sunlight better than a commercial formulation. Preliminary work conducted with viruses suggests that not all the matrix-forming ingredients perform the same with respect to protection of viral activity but that some may be suitable. Formulation of fungi and nematodes present far different challenges than viruses or bacteria in that humidity and free moisture play an essential role in survival of the infectious particle until contact with a suitable host. Work is currently in progress with these two organisms.

21-026

Insecticidal activity of isolates of *Bacillus thuringiensis* on larvae and adults of *Bactrocera oleae* Gmelin (Dipt. Tephritidae)

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Several hundreds of *Bacillus thuringiensis* isolates have been obtained from the proximity of olive groves and in olive presses in several areas of Greece, Sardinia (Italy) and Spain. Most of the isolates have been bioassayed for toxicity against *Bactrocera oleae* (Gmel.) (Dipt. Tephritidae) (larvae and adults). The levels of biotoxicity detected differ significantly amongst the isolates. The toxicity against larval stage of *B. oleae* does not always correspond to the biotoxicity against the adults. Gene analysis of the most toxic strains for most of the known toxin genes has revealed that several isolates are not harboring any of the known delta-toxin genes; these strains therefore could contain novel toxin genes.

21-025

USE OF MICROSPORIDIA FOR MANAGEMENT OF MOSQUITOES

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Tremendous progress has been made in recent years to resolve the complex relationships between pathogenic microsporidia and their mosquito hosts. Recent attention has focused on species of heterosporous (polymorphic) microsporidia belonging to the genera *Amblyospora* and *Edhazardia*. Heterosporous microsporidia are characterized by intricate life cycles involving multiple spore types responsible for horizontal (per os) and vertical (transovarial) transmission. These microsporidia affect two generations of the mosquito host, some require an obligate intermediate host and they are generally very host and tissue specific. Microsporidian parasites detrimentally impact mosquito populations by 1) causing high levels of larval mortality following vertical transmission and 2) through a reduction in the overall fitness of the infected adult following horizontal transmission. This information has been used to evaluate these parasites as biocontrol agents of mosquitoes and to examine their role in preventing the transmission of disease to man and animals.

21-027

RECENT ADVANCES IN THE USE OF ENTOMO-PATHOGENIC FUNGI FOR PEST CONTROL IN FIELD CROPS

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Use of pathogenic fungi for biological control of insect pests infesting field crops has been pursued for more than a century, with only limited commercial success. Important recent advances, however, have led to a steadily increasing interest in product development, and since the beginning of this decade, a number of hyphomycete-based mycoinsecticide products have been patented and/or registered worldwide for use against a variety of insect pests. The long-term commercial potential of these new fungal products is being carefully evaluated by an agricultural community seeking softer alternatives to broad spectrum synthetic chemicals insecticides. In this presentation we discuss promising new fungus production and formulation technologies and application methods and strategies that are translating into greater and more consistent efficacy under field conditions. *Beauveria bassiana*, one of the most ubiquitous and extensively studied of the entomopathogenic fungal species, is the active agent in many of the products currently under development. The present status of development of this fungus for control of *Bemisia tabaci* (Homoptera: Aleyrodidae) and *Leptinotarsa decemlineata* (Coleoptera: Chysomelidae) is reviewed to illustrate current progress and prospects for successful incorporation of fungal agents into diverse integrated pest management systems.

21-028

FUNGAL PATHOGENS FOR INSECT CONTROL IN FRUIT TREE ORCHARD SYSTEMS

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Use of fungal pathogens for control of insect pests in orchards has been a much neglected area of research. Microbial insecticides are considered to be impractical for insect pests that directly feed on the fruit because of low damage thresholds, thus, chemical insecticides have been favored. However, microbial insecticides can be a suitable alternative to chemical insecticides for controlling insect pests that do not directly feed on the fruit, but instead, feed primarily on tree leaves. Considerable amounts of chemical insecticides are used against these pests because they lower yields by reducing net photosynthesis or by indirectly damaging the fruit by insect honeydew. Herein, I review past uses of fungal entomopathogens in orchard systems and report my efforts in using fungal pathogens for controlling pear psylla, *Cacopsylla pyricola* Foerster, a serious indirect pest of pear. Based on laboratory bioassays, isolates of *Beauveria bassiana* and *Paecilomyces fumosoroseus* were chosen for field studies. Various formulations of conidia were applied at 4.9×10^{13} /hectare at different times of the season from 1993-1995 and nymphal mortalities were monitored over a 7-9 day period. Mortalities peaked 5-7 days after application and varied from 20-70% mortality, depending on the formulation and environmental conditions. Spore survival was not enhanced by formulation and spore mortalities were >70%, 24 hours after application. The potential of fungal pathogens for controlling this pear psylla will be discussed in light of these results.

21-030

RECENT ADVANCES IN FORMULATION AND APPLICATION OF FUNGAL PATHOGENS FOR CONTROL OF AFRICAN LOCUSTS

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Since the massive desert locust upsurge of the late 1980s, national and international research projects were set up to develop environmentally safe locust control methods. Investigations on fungal insecticides were mainly fostered by the German GTZ-BBA project, the international LUBILOSA project and the USAID project at the Montana State University. During these studies, the fungi *Beauveria bassiana*, *Metarhizium anisopliae* and, especially, *M. flavoviride* were the most promising candidates for biocontrol of African locusts. Recently, important progress has been made in the mass production of infective fungal propagules, their formulation and their application in the field. Conidia are produced on the surface of solid or liquid media, whereas blastospores or submerged conidia are produced in liquids in fermenter systems. The LUBILOSA project developed an oil-based formulation of conidia of *M. flavoviride* for use in controlled droplet application technology at ultra-low-volume (ULV) rates. Within the GTZ-BBA project, we developed an experimental product based on spray-dried blastospores of *M. flavoviride*. These show an excellent shelf life up to two years at 5 °C and they can be suspended both in water and oil. In a comparison of different formulations in semi field trials in Mauretania, a water-based formulation (20% molasses + 80% water) of spray-dried blastospores caused a high mortality against larvae and adults of *Schistocerca gregaria* after ULV application. - The importance of efficient and stable formulations, their compatibility with standard application methods and the integration of a mycoinsecticide in a locust control strategy will be discussed.

21-029

DEVELOPMENT OF *METARHIZIUM ANISOPLIAE* FORMULATIONS WITH NEW DELIVERY SYSTEMS FOR THE CONTROL OF CRUCIFER PESTS.A.I. David-Henriet, B.J. Pye, L. Ibrahim¹, T.M. Butt².

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Oilseed rape (OSR) is one of the most important oleaginous crops in Europe. New microbial based strategies are under development to reduce the input of chemical pesticides in this crop. Isolates of the entomogenous *Metarhizium anisopliae* have been identified which are highly pathogenic for several crucifer pests, including *Psylliodes chrysocephala*, *Lipaphis erysimi* and *Myzus persicae*, yet comparatively harmless to honey bees.

Conidia of *M. anisopliae* were formulated in different oil types (mineral, paraffin and vegetable). The best results were obtained using a paraffinic oil carrier (Shellsol T at 70%) and with either mineral oil (Ondina-El at 30%) or a vegetable oil (e.g., Sunflower oil at 30%, groundnut oil, soya oil...). Applications using these formulations resulted in up to 70% mortality of adults *Phaedon cochleriae* (using as a model to compare with *Psylliodes chrysocephala*) five days post-inoculation.

Ultra low volume application of conidia/spore formulations were applied using the APE 80, electrostatic delivery system developed at Rothamsted. Much improved deposition of inoculum was obtained in field trials with the electrostatic delivery system compared with conventional hydraulic spraying systems.

A comparison was made between conventional aqueous formulations of conidia and the oil formulated conidia. The latter consistently gave increased insect mortality over the same time period.

21-031

EFFECTS OF THE *BEAUVERIA BASSIANA*-BASED MYCOINSECTICIDE MYCOTROL® ON A PARASITOID OF *BEMISIA TABACI*W. A. Jones and T. J. Poprawski¹

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Laboratory tests were conducted to determine if *Beauveria bassiana* (at 1000 spores/mm² of strain GHA) kills immature *Eretmocerus* sp. at 1, 2, 3, 9, and 13 d after parasitization. Treatments were: fungus in an aqueous carrier containing 0.02% Tween 80 surfactant, 0.02% Tween 80 solution without fungus, and water alone (control). Spore applications were made to host nymphs on excised sweet potato leaves rooted in hydroponic solution.

The results generally demonstrated that following parasitoid egg hatch, the fungus was unable to colonize parasitized host nymphs. For the 9- and 13-d-old treatments, no infection of parasitized hosts was successful. Rates of successful parasitism among hosts sprayed at day 3 were similar to those in the controls. Rates of infection at day 1 and 2 were similar to that for unparasitized host cohorts. Spores applied to the cuticle of parasitized nymphs germinated normally and penetration pegs were present. Longevities of adults surviving the 3- and 13-d-old fungus sprays were significantly lower than those surviving the Tween 80 solution, but no difference was measured for the 9 d treatment. Fecundity between mated females surviving the 13 d treatments of fungus and Tween 80 was not significantly different, producing 148.1 and 164.2 mean progeny per female, respectively.

Field tests of Mycotrol in vegetables demonstrated that application of the fungal pathogen against whiteflies is less lethal to foraging parasitoids than insecticides.

21-032

GEOGRAPHICAL AND SPATIAL DISTRIBUTIONS OF THE ENTOMOPHAGOUS AND MYCETOPHAGOUS NEMATODE, *CONTORTYLENCHUS GENITALICOLA*, IN THE CERAMBYCID HOST, *MONOCHAMUS ALTERNATUS*, POPULATIONS

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Contortylenchus genitalicola Kosaka and Ogura is an entomophagous and mycetophagous nematode parasitizing the Japanese pine sawyer, *Monochamus alternatus* Hopc. *M. alternatus* is a serious forest pest because it transmits the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhner), a causal agent of severe pine wilt disease in eastern Asia. *C. genitalicola* is considered a candidate biological control agent against *M. alternatus* since this nematode reduces reproductive potential of its host.

Geographical and spatial distribution of *C. genitalicola* in *M. alternatus* populations was investigated to know the nematode parasitism more clearly. *C. genitalicola* was found in adult *M. alternatus* populations from various parts of Japan. For instance, *C. genitalicola* was isolated from *M. alternatus* adults in Kumamoto pref., southern part of Japan, and in Akita pref., northern limit of the insect distribution. Prevalence of *C. genitalicola* in *M. alternatus* populations from Kamisu, Ibaraki pref., central part of Japan, during 5 years was about 5 % in each year. Spatial distribution of *C. genitalicola* in *M. alternatus* populations from Kamisu was aggregated or over-dispersed and the distribution fitted to the negative binomial.

21-034

STEINERNEMA KUSHIDAI, A NOVEL BIOLOGICAL CONTROL AGENT FOR THE SOIL LIVING COLEOPTERAN BEETLES

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S. kushidai, an entomopathogenic nematode, which was found in Japanese soil in 1984, showed the high activity against various Coleopteran beetles. In Japan the Coleopteran beetles are a major group of insects that feed on the roots of economically important crops, such as turfgrass, sweet potatoes and peanuts. We could maintain the nematodes in highly active form and make progress in research areas such as mass-fermentation and formulation.

S. kushidai has a wide insecticidal spectrum against beetles dwelling in Japanese soil. The nematodes showed high activity between 20-30°C, which is similar to larvae's general feeding behavior. This suggests that nematodes generally penetrate the larval body through the mouth.

We applied the formulated nematodes into turfgrass field of golf courses at the rate of 250,000-500,000 nematodes/m² and could observe high mortality of the beetles after 2-4 weeks post treatment. The nematodal density in the soil increased 6 times during this period, indicating the occurrence of secondary infection in the field.

21-033

POTENTIAL FOR USE OF ENTOMOPATHOGENIC NEMATODES AGAINST TEPHRITID FRUIT FLIES

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Caribbean fruit flies, *Anastrepha suspensa* (Loew), are susceptible to infection by heterorhabditid nematodes. Laboratory studies have yielded infected larvae, pupae, and adults. The larval stage, however, would be the most likely stage to target in a biological control program as it is most susceptible to infection and infective juveniles can easily exit the host for recycling in a soil environment. Adequate soil moisture is critical for optimal activity of soil dwelling entomopathogenic nematodes. The use of entomopathogenic nematodes as a component of a biorational IPM program (such as use with food based attractants) against these pest insects warrants further investigation.

21-035

EFFICIENCY OF *HETERORHABDITIS BACTERIOPHORA* POINAR (NEMATODA: HETERORHABDITIDAE) UNDER INSECTICIDE-STRESS IN PARASITIZING *SPODOPTERA LITURA* FABR. (LEPIDOPTERA: NOCTUIDAE).

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The present work is an attempt to assess the biocontrol potential of *Heterorhabditis bacteriophora* against *Spodoptera litura* larvae, which are often treated with malathion for their control in the fields. This insecticide mostly washed down in the soil. Motility of infective juveniles (IJs) of *H. bacteriophora* was recorded in terms of number of undulations per minute to ascertain their vitality after exposing to various sublethal concentrations of malathion. The IJs showed incomplete undulations as well as decreased motility with the increase in malathion concentration. Infectivity tests were conducted using filter paper bioassay. The reduced parasitization rate with respect to *S. litura* larvae with increasing concentrations of malathion manifested negative correlation between IJs of *H. bacteriophora* and various concentrations of malathion. Malathion treated larvae of *S. litura* were also found to be suitable hosts for *H. bacteriophora*. The potential of *H. bacteriophora* is discussed towards insect pest suppression in IPM.

21-036

AGGRESSIVENESS AND SPECIFICITY OF ARGENTINEAN *STEINERNEMA* AND *HETERORHABDITIS* SPECIES.

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Entomopathogenic nematodes that belong to *Steinernema* and *Heterorhabditis* species, are promising agents in the control of insect pests. Recently, several isolates characterized by a higher infectivity have been detected in Argentina: *S. rara*, *S. glaseri* (respectively from Noetinger and Los Chorrillos, Córdoba) and *H. bacteriophora* (from Rio Negro). In order to know the host range, experiences were carried out in laboratory conditions to evaluate the aggressiveness and specificity of these autocton isolates. Different species of insects (n = 33) (larvae, nymphs and/or adults) were put in contact with infectives larvae. The mortality caused by the nematode and its development were registered. According to the species of nematodes, the insect class, its stage, and its feeding habits, the infectivity and degree of development of nematode were different. All the lepidopterans considered (agricultural pests) were attacked and they appeared as excellent hosts. In contrast, the aquatic dipterous were resistant. All species of coleopterans and homopterous considered were attacked except *Astylus atromaculatus* and *Aphis gossypii*. The hematophagous insects were rarely infected, and the development of nematode were limited. Generally, the larvae appeared more susceptible than the adults. Considering the results obtained the isolates tested would be considered in the biological control of harmful insects.

21-038

INFLUENCE OF ENTOMOPATHOGENIC NEMATODES ON THE PREDATORS BEETLES (CARABIDAE)

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The effect of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* on mortality of *Bembidion properans*, *B. lampros*, *Pseudophonus pubescens*, *Pterostichus vulgaris* and *P. cupreus* was studied in the laboratory conditions. Invasive larvae in dose 1000 nematodes per insect were applied into the soil in plastic cups, in which the imagines of Carabidae were separately kept. Also effect of the food previously contacted with nematodes on the mortality of predators beetles was simultaneously studied. Both nematodes introduced into the soil and food infected with nematodes and consumed by Carabidae increased the mortality of imagines after three weeks. Semi - field trials showed that *Bembidion lampros* and *B. properans* were little susceptible to these nematodes. In field trials with using *S. carpocapsae* against two species of carabids from annual legumes *B. properans* and *Pterostichus cupreus*, most often captured into pitfall traps, no effect was found in case of different doses of invasive larvae on the soil surface.

21-037

PREVALENCE OF HIERARCHICAL RESPONSES TO HOST CUES AMONG ENTOMOPATHOGENIC NEMATODE SPECIES

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The response of entomopathogenic nematodes to host cues is closely linked with their foraging strategy. Lewis et al. (1995; Parasitology 110: 207-213) found that *Steinernema carpocapsae*, an ambushing forager, responds to host chemicals only when they are encountered in a specific order: contact with host cuticle stimulates attraction to volatile cues emanating from the host. In the same study *S. glaseri*, a cruise forager, was found to respond to either contact or volatile cues with or without a primer stimulus. *Steinernema feltiae* responded in an intermediate manner to combinations of host cues. *S. feltiae*'s responses to host cues offered individually was stronger than that of *S. carpocapsae* but weaker than *S. glaseri*, and exposure to host cuticle increased attraction to volatile cues. In the present study, the response to hierarchical orders of hosts cues is examined for several species of entomopathogenic nematodes including *S. scapterisci*, *S. riobravensis* and *Heterorhabditis bacteriophora*. Infective stage juveniles were tested for their attraction to host volatile cues either immediately after exposure to contact with *Galleria mellonella* cuticle for 30 minutes or without this contact. This study had two objectives. The first was to test the hypothesis that all nematode species that nictate, an ambushing behavior where the nematode elevates 95% of the body from the substrate, should respond to host cue hierarchies similarly to *S. carpocapsae*. The second objective was to examine more nematode species with "intermediate" foraging strategies (*S. riobravensis*, for example) to determine the commonality of the hierarchical response.

21-039

EFFECT OF INSECT HOST ON SUBSEQUENT INVASION OF ENTOMOPATHOGENIC NEMATODES

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The phenomenon that only part (10-40%) of the nematode population invade the target host has been recorded in many studies. It has been mainly ascribed to differences in the ability of individual nematodes to infect. In the present study the effect of an infected host, the wax moth *Galleria mellonella*, on subsequent infection of the entomopathogenic nematodes *Steinernema carpocapsae* Mexican, *S. riobravensis* and *S. feltiae* was evaluated.

The invasion rate of the three nematode species to a non-infected host was reduced by 40-60% after pre-exposure to infected hosts. These nematodes regained their full invasion potential after they were rinsed with water. Invasion into insects which were previously injected with nematodes was significantly reduced by 60-80% 6-9 h after injection. The reduction in subsequent invasion due to the initial infection was nematode species-specific. This phenomenon was also observed with other lepidopteran pests (*Helicoverpa armigera* and *Spodoptera littoralis*).

The data indicate that the initial infection by entomopathogenic nematodes induced the release of a substance which reduced the subsequent invasion. The chemical and biological characteristics of this substance are currently under investigation.

21-040

EFFECTS OF THE TSETSE DNA VIRUS ON THE ULTRASTRUCTURE AND FUNCTIONS OF THE ACCESSORY REPRODUCTIVE GLAND IN THE HOST FLY *GLOSSINA MORSITANS CENTRALIS* (DIPTERA: GLOSSINIDAE)

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Third instar *Glossina morsitans centralis* larvae were infected with the tsetse DNA virus by microinjection and at emergence adult males were fed every other day for 8 days and comparative observations made on some physical parameters, functions and the ultrastructure of the accessory reproductive glands (ARG) in infected and control flies. The ARG from control flies were found to be milky in appearance while those from virus infected flies were mostly transparent. The ARG from virus infected males had significantly smaller diameters ($F=200.4$, $P<0.0001$) than those of controls.

The uteri of females mated to virus infected males were either empty or contained partially formed spermatophore while uteri of females mated to control males contained fully formed spermatophore.

Histological studies of ARG tissues from virus infected males revealed some lesions in the epithelial cell layer characterised by degeneration of cytoplasmic organelles, vacuolation in the cytoplasm and detachment of the muscle layer from the basal plasma membrane.

21-042

ELECTRON MICROSCOPY OF A MALAYSIAN ISOLATE OF BACULOVIRUS FROM *SPODOPTERA LITURA* FAB.

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The morphology and histopathology of a nuclear polyhedrosis virus (NPV) isolated from *Spodoptera litura*, the polyphagous leaf-eating caterpillar, were studied by transmission electron microscopy. The purified polyhedra had a spherical to oval outline measuring about 1.9-2.8 μm in diameter. Within the proteinaceous capsule or polyhedra many virions were observed and within each virion more than one nucleocapsid per envelop that appeared to be the MNPV type of the genus *Nucleopolyhedrosis*. Treatment with chemical detergent resulted in the release of the rod-shaped nucleocapsid measuring about 500 nm in length and 110 nm in diameter. Development of the NPV after 3 days post feeding of the polyhedra inclusion bodies (PIBs) was shown in thin sections of the infected larva fat body tissues. A number of PIBs were found in the nucleus with various size and shape. These were the occluded phenotypes which matured in the nuclei and contained many enveloped virions. Each of the virion supported several nucleocapsids, some as many as six. At later stages between four to six days post feeding of the PIBs the virogenic stroma gave rise to virus nucleocapsids which were single nucleocapsids. These were the second phenotype of virions that budded from the plasma membrane into the cytoplasm of infected cells.

21-041

TRANSMISSION DYNAMICS OF AN INSECT-VIRUS INTERACTION

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Information on the factors which influence virus transmission in insect populations can contribute to the optimal use of these agents as biopesticides as well as to the understanding and prediction of virus epizootics. However, quantitative studies on virus transmission are rare, and tend to be concentrated on forest pests.

In a series of field trials, the transmission dynamics of an agricultural pest (*Mamestra brassicae* L., Lep: Noctuidae) and its nucleopolyhedrovirus (Family *Baculoviridae*) was investigated. Densities of host and pathogen were manipulated in cabbage plots. Virus acquisition was estimated by destructive sampling at periodic intervals. Mortality increased with time of exposure to inoculum and with densities of host and virus. Times to death for each treatment and the transmission rates were estimated using mathematical models.

21-043

PATHOGENICITY AND CHARACTERISTICS OF *SPODOPTERA LITURA* NUCLEAR POLYHEDROSIS VIRUS FROM PENINSULAR MALAYSIA

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Spodoptera litura (Fabricius) is a cosmopolitan polyphagous insect pest of many food crops and tobacco. As in most other parts of the tropic, these armyworms are subject to a disease caused by a nuclear polyhedrosis virus (NPV) infection in the field. In Malaysia, no record of such an infection has been reported. Only recently, however, an epizootic of NPV disease occurred in *S. litura* population attacking tobacco crop in Kelantan, Malaysia. The incidence indicated that natural control of *S. litura* by NPV was operating in the tobacco ecosystem in the absence of a prophylactic insecticidal treatment. Though the impact of the disease on the *S. litura* population has yet to be known, NPV has the potential to be used as a control agent to be incorporated in management of *S. litura* in Malaysia. A study on the characteristics of the NPV was carried out. The result shows that the virus was pathogenic and specific to its host. It caused 96% larval mortality within a period of 10 days at a dosage of 6×10^8 PIBs/larva and had no adverse effect on Lepidoptera, *Spirama retorta* Clerck (Noctuidae) and bagworm *Pteroma pendula* Joannis (Psychidae). The length of the virus DNA was in the range of 109 to 151 kilobase pairs. This was identical to those of DNAs in other *Spodoptera* viruses; *S. exigua* NPV and *S. frugiperda* NPV.

21-044

VIRAL EPIZOOTICS INDUCED BY THE GRANULAR VIRUS IN *PLODIA INTERPUNCTELLA* HB.

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Plodia interpunctella Hb. is a major polyphagous storage pest attacking grain, dried fruits and especially those included in chocolate, causing severe damages.

Controlling this pest with chemicals is inadequate, therefore alternative non-toxic means to regulate population densities, safe to man and environment unpolluting seem to be useful.

One of the most efficient option could be the use of baculoviruses, having no negative influences on other components of storage biocenoses and to human safeguard.

Till now, two groups of viruses have been detected in *P.interpunctella*:

- the nuclear polyhedrosis virus (NPV);
- the granulosis virus (GV).

In *P.interpunctella* populations occurring in Romania, laboratory-reared for several years on suitable diets, only the granular virus (GV) has been detected. Trials have demonstrated that the larval mortality in L-3 and L-4 instars caused by this virus at 26-27°C reached 90-95%. Peak of mortality caused by viral infections nearly in all larval instars has been noted 8-9 days after the infection start.

These data suggest a prospective to elaborate a new viral product destined to regulate population densities in *P.interpunctella*.

21-046

FIELD-BASED RISK ASSESSMENT OF GENETICALLY MODIFIED BACULOVIRUSES IN TARGET AND NON-TARGET HOSTS

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Insect pathogens show great potential as pest control agents, however, their use has been limited because of their relatively slow speed of action. Genetic engineering is perceived as a means whereby their insecticidal potential can be enhanced. Field trials have shown that the application of a baculovirus which expresses an insect-selective toxin results in a more rapid kill and in reduced crop damage as compared to the unmodified wild type. Before these organisms are released into the environment on a wide scale any risks attached to their release must be assessed. Using a virus expressing an insect-selective toxin derived from a scorpion as a model system, contained field trials were run to compare the effect of the recombinant virus and its parent wild-type on lepidopteran hosts which varied in susceptibility. Virus was introduced in two ways; as a spray (like an insecticide application) and as infected cadavers (the major route for transmission in insect larvae). As expected, virus-induced mortality was much lower in the less susceptible host species in both treatments. However, in the spray trial the mortality induced by both viruses was equal whereas in the cadaver trial the scorpion toxin virus produced significantly less mortality than the wild-type. This is because the larvae infected with the recombinant virus become paralysed and tend to fall off the crop plant, thereby removing the inoculum from other healthy larvae. These results indicate that transmission of these toxin-expressing baculoviruses will be considerably reduced in the field.

21-045

FORMATION OF OCCLUSION BODIES OF NUCLEAR POLYHEDROSIS VIRUSES IN THE INFECTED CELLS OF *MALACOSOMA NEUSTRIA* L. (LEPIDOPTERA: LASIOCAMPIDAE).

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Baculoviruses rather rarely cause epizootics in pest populations under Latvian climatical conditions. Nuclear polyhedrosis viruses have been isolated from 7 insect species. *Malacosoma neustria* L. has been used as a model insect in the cytological researches.

Infectious process of *M. neustria* caused by the Latvian isolate of *Malacosoma neustria* nuclear polyhedrosis viruses (Mn NPV) was investigated. The LD50 values were 75 and 985 polyhedral occlusion bodies (polyhedrae) per larvae of the second and third instars, respectively. Tissue and cells in the latest stage of the disease after per os infection content a large amount of viral polyhedrae. Electron microscopy shows that at the 6th day after infection polyhedrae, multiple virions and nucleocapsids are visible in the nucleus of gut epithelial and fat cells. The dimension of polyhedra is 900-5000 nm. Rod-shaped nucleocapsids are 220 - 360 nm in length and 30 - 60 nm in width. Polyhedrae of the MnNPV Latvian isolate content multiple virions, which content 2-6 nucleocapsids each. The development process of the membrane of virions and polyhedrae has been investigated. In particular cases polyhedrae have been observed with necrotical changes.

Obtained results of the natural way of polyhedrae formation will give the possibilities to estimate the influence of substances used in virus preparation, on this process. The influence of inhibitors and stimulators will also be determined.

21-047

COMPARISON OF INFECTIVITY, TIME-TO-DEATH AND PRODUCTIVITY BETWEEN THE WILD TYPE AND RECOMBINANT BACULOVIRUSES IN HOSTS DIFFERING IN SUSCEPTIBILITY

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A genetically modified baculovirus encoding a scorpion toxin gene, which reduces the survival time of its host, was compared with its parental wild type clone in laboratory assays. Early second instar larvae of *Trichoplusia ni* (a permissive species) and *Mamestra brassicae* (a semi-permissive species) were infected at different concentrations with both viruses. LC50 values did not differ significantly between viruses, but were lower for *T. ni* than for *M. brassicae*. Survival time and virus yield were lowest when *T. ni* was infected with the recombinant virus. In all treatments, the yield:weight relationship was shown to be a complex interaction between dose, virus type and host species, suggesting that virus yield cannot be estimated from weight alone. Although the recombinant virus reduced the mean time to death, yield of virus was reduced as a consequence. This has implications for the fitness of the virus in the field.

21-048

BEHAVIOURAL CHANGES IN BACULOVIRUS-INFECTED *MAMESTRA BRASSICAE* L. (LEP: NOCTUIDAE) LARVAE AND THEIR RELEVANCE TO THE SPATIAL SPREAD OF INOCULUM

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The effect of baculovirus infection on the mobility of 2nd and 4th instar *M. brassicae* larvae was investigated in the laboratory and field. BV infection clearly altered larval mobility, with the changes in behaviour varying with the timecourse of infection. Diseased larvae moved 3 to 5 times further than healthy ones during the middle stages (4 days) of infection. By the 7th day post-infection diseased larvae were less mobile than healthy counterparts. The same pattern of modified behaviour was observed in both instars.

In the field, infected larvae tended to die on the apex of the cabbage leaves. Bioassay of the leaves showed that there was a linear decrease of inoculum from central to peripheral plants within the plots, which occurred to the same extent for 2nd and 4th instars. Leaves from plots where infected 4th instar larvae had been introduced had higher inoculum density than plots with 2nd instars.

21-050

INTERACTION BETWEEN *APANTELES KARIYAI* WATANABE (HYMENOPTERA: BRACONIDAE) AND AN ENTOMOPOXVIRUS IN THEIR HOST *PSEUDALETIA SEPARATA* (WALKER) (LEPIDOPTERA: NOCTUIDAE)

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The common armyworm *Pseudaletia separata* is subjected to control by its sympatric natural enemies *Apanteles kariyai* and an entomopoxvirus (PsEPV) in its natural habitat. The possible interactions between these control agents were studied in the laboratory to examine the effect of PsEPV infection of the host on parasitoid survival.

The fate or survival to maturity of *A. kariyai* in entomopoxvirus infected *P. separata* larvae was affected by the time of parasitization prior to the inoculation of the host with PsEPV.

The number or proportion of parasitoids that survived and emerged to form cocoon increased as the period between oviposition and virus infection increased. Parasitoids could not kill host larvae and emerge as adult wasps if they develop in host that were exposed to PsEPV in less than 5 days after parasitization.

The development of the parasitoid *A. kariyai* within PsEPV-infected *P. separata* was adversely affected and this effect occurs prior to host death.

21-049

NUCLEOTIDE SEQUENCE OF FUSOLIN GENE OF *ANOMALA CUPREA* ENTOMOPOXVIRUS (AcEPV)

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A larva of the cupreous chafer, *Anomala cuprea* is a major pest in many crops and forest trees in Japan. A pathogenic entomopoxvirus of this insect, AcEPV, may be considered as a potential biocontrol agent. In this study, the nucleotide sequence of major protein of spindle, fusolin, was investigated. The molecular size of fusolin was estimated as 46 kDa from the analysis of SDS-PAGE. When PCRs (polymerase chain reactions) were tried using the many primer sets constructed from the sequence data of the fusolin gene of *Melolontha melolontha* entomopoxvirus (MmEPV) (Gauthier *et al.*, 1995), only one primer set was available to amplify a part of AcEPV fusolin gene. This 0.3 kb PCR product was sequenced and a new primer set was constructed from the resultant data for inverse PCR elongating towards both upstream and downstream of the PCR product. The inverse PCR product was cloned into pBluescript II and its deletion series were sequenced. The homology (matching percentage) between AcEPV and MmEPV fusolin gene was about 75%, which was lower than that (ca. 90%) of spheroidin homology. From the result, the biological importance of spindle was thought to be smaller than the spheroid, though the biological significance of the former was remained unknown.

21-051

AN INVESTIGATION INTO THE PERSISTENCE AND INTERACTION OF TWO VIRUSES OF *AGROTIS SEGETUM* (D.&S.) (LEPIDOPTERA: NOCTUIDAE) IN THE DEVELOPMENT OF LONG TERM CONTROL STRATEGIES.

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The common cutworm, *Agrotis segetum* (Denis & Schifferrmüller), is a highly polyphagous and widespread pest. It is one of a number of noctuid moths whose larvae live mainly in the soil during later instars, feeding by severing stems and boring into roots and tubers. Its soil-dwelling behaviour makes it a difficult target for chemical sprays and larvae may remain undetected until severe damage has occurred.

Baculoviruses represent an important and effective alternative for cutworm control. The soil acts as a reservoir for virus between larval outbreaks and protects inclusion bodies against environmental degradation. Larvae may ingest inclusion bodies from this site, and so by maximising soil persistence, immediate effective control may be achieved in subsequent generations. In a two-year field trial, it was shown that a granulovirus of *A. segetum* showed greater persistence than a nucleopolyhedrovirus or a combination of the two and that no interaction occurred in mixed infections.

21-052

INSECT SMALL RNA VIRUSES OFFER NEW STRATEGIES IN PEST CONTROL.

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Insects are host to a number of small non-enveloped RNA viruses which offer new approaches to pest control. Modern techniques of genetic engineering allow the specificity, simplicity and unique properties of these viruses to be exploited by producing them in non-host systems such as in transgenic plants. We are exploring the potential of a recently characterised tetravirus, *Helicoverpa armigera* stunt virus (HaSV), for the control of heliothine pests including the cotton bollworm. Upon ingestion by neonate larvae, this virus is specific for cells of the midgut and rapidly causes the larvae to cease feeding, so that they become stunted and die. Its genome consists of only 3 genes on 2 strands of RNA. We have constructed synthetic virus genes designed to produce infectious virus particles in non-hosts such as plants or insect cells infected using a baculovirus vector. Initial data from transgenic plants indicate the efficacy of this approach: assembly of infectious, pest specific virions in the plants is able to protect them against feeding damage.

21-054

DISTRIBUTION AND ABUNDANCE OF SOIL-BORNE NUCLEAR POLYHEDROSIS VIRUSES (NPV: BACULOVIRIDAE) INFECTING THE COTTON BOLLWORM, *HELICOVERPA ARMIGERA* (HÜBNER) (LEPIDOPTERA NOCTUIDAE) IN EASTERN AUSTRALIA

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Data on the natural abundance of insect pathogens in the soil environment is scarce even though the soil is thought to play an important role in the long-term persistence and transmission of such pathogens in host populations. A bioassay method employing the direct incorporation of soil samples into an insect diet has provided a rapid and convenient method for estimating the loading of soil-borne *Helicoverpa* NPVs in the Australian agro-ecosystem.

Lethal concentration curves for HaSNPV against 24 hour old *H. armigera* larvae have been constructed for different rates of soil incorporation (0.005g soil/ml diet at the lowest rate up to 0.25g soil/ml diet) This method provides consistent detection of virus down to a level of <50 PIBs/g soil. In addition, amplification of NPV DNAs from infected cadavers using the polymerase chain reaction (PCR) technique, followed by restriction endonuclease analysis of the PCR product, allows rapid genotyping of the naturally occurring isolates. Using selected oligonucleotide primers, separation of very closely related NPVs (<2% nucleotide divergence) has been possible. Data obtained from soil-NPV surveys conducted in eastern Australia, with samples from a diversity of cultivated and non-cultivated sites, will be presented.

21-053

PERSISTENCE, DISPERSAL AND TRANSMISSION OF A NUCLEAR POLYHEDROSIS VIRUS (NPV: BACULOVIRIDAE) INFECTING THE COTTON BOLLWORM, *HELICOVERPA ARMIGERA* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE) IN AUSTRALIAN COTTON

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Field and laboratory studies employing wild-type *Helicoverpa* NPVs are providing the basis for the design of environmental monitoring protocols for the release of genetically modified viruses. Using simple bioassay approaches for virus detection combined with a PCR-REN technique for virus identification, experiments to date have focused on three major aspects of insect-pathogen ecology: persistence, dispersal and transmission.

In the first case, data on the rate of virus decay in the cotton leaf canopy and in the soil beneath the canopy have shown that NPV half-life values are strongly dependent on the position of virus in the canopy as well as crop growth stage. The pattern of virus dispersal across the field surface following irrigation was shown to follow a logarithmic fall off in NPV activity with distance from virus source. Soil cores from field plots and laboratory experiments using soil columns indicated little propensity for NPV to disperse vertically through the soil profile. In the virus transmission study, the importance of the soil as a virus reservoir for the translocation of NPV to host feeding sites and subsequent transmission in the host population was addressed. The significance of these data and of the approaches adopted will be discussed with respect to the environmental impact assessment of genetically modified insect viruses.

21-055

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF MICROSPORIDIA FROM FIRE ANTS

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Two species of imported fire ants, the black imported fire ant *Solenopsis richteri* and the red imported fire ant *S. invicta*, were introduced into the United States from South America in the early part of this century. *Solenopsis richteri* has a limited distribution but *S. invicta* has spread throughout the Southern United States. It is considered a major agricultural and urban pest in the US but not in its home lands of South America. This may be due, in part, to the lack of natural enemies and competitors in the US. Expeditions to South America seeking natural enemies have yielded two microsporidia, *Thelohania solenopsae* and *Vairimorpha invicta*, both described from *S. invicta* in Brazil. Morphologically similar microsporidia (*Thelohania* sp. and *Vairimorpha* sp.) have been reported from a high proportion of *S. richteri* collected in Argentina. Their role as biological control agents for *S. invicta* in the US is being investigated but transmission trials with these microsporidia have been unsuccessful. A fundamental question, therefore, is to determine if *T. solenopsae* and *V. invicta* are conspecific with *Thelohania* sp. and *Vairimorpha* sp. Furthermore, since microsporidia with several spore types are known, we investigated the possibility that *Vairimorpha* sp. and *Thelohania* sp. could be different phenotypes of the same species. Morphological characterization using light and electron microscopy together with molecular analysis of the 16S rRNA gene sequences were employed to compare the species.

Gross morphology and ultrastructural comparisons could not distinguish *T. solenopsae* from *Thelohania* sp. or *V. invicta* from *Vairimorpha* sp. Comparison of the 16S rRNA gene sequences between *T. solenopsae* and *Thelohania* sp. demonstrated a sequence similarity of more than 99%. Based on this data, *T. solenopsae* and *Thelohania* sp. are conspecific or represent two subspecies. Molecular data are not available but required for *V. invicta* in order to determine whether *V. invicta* and *Vairimorpha* sp. are conspecific or represent two subspecies.

The two *Thelohania* spp. and two *Vairimorpha* spp. are distinct from each other morphologically. *Thelohania* sp. and *Vairimorpha* sp. from *S. richteri* share a sequence similarity of only 63%. These data show that *Thelohania* sp. and *Vairimorpha* sp. are distinct species in separate genera.

21-056

THE CHARACTERIZATION OF *NOSEMA LITURAE* ISOLATED FROM TAIWAN

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The epizootic disease causing death in *Spodoptera litura* (Lepidoptera: Noctuidae) occurring in Taiwan at fall season in every year was shown to be caused by *Nosema liturae*. Structural properties of this microsporidia were examined by electron microscopy, polyacrylamide gel electrophoresis, immunoblot assay and restriction endonuclease analysis and compared with other *Nosema* isolates from other pests, *S. exigua*, *Helicoverpa armigera*, *Plutella xylostella*, and *Pieris* spp., and with *Pleistophora anguillarum* from eel. The results had shown that no significant difference between *N. liturae* and other *Nosema* isolates, but no any relation with *P. anguillarum*, was found. Bioassays of *N. liturae* gave LD₅₀ values 1.1×10^4 for third-instar larvae of *S. litura* and was not found to differ significantly from other *Nosema* isolates. We suggest that five isolates of *Nosema* spp. are closely related genetically.

21-058

INFECTION AND DEVELOPMENT OF *NOSEMA LITURAE* (MICROSPORA) IN IPLB SF-21AE CELL LINEC. H. Wang¹, S. J. Tsai¹, C. F. Lo²

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Tissues collected from tobacco cutworm (*Spodoptera litura* Frabricius) experimentally infected with *Nosema liturae* were cocultivated with IPLB SF-21AE cells in TNM-FH medium. The in vitro cultivation system of this microsporidian was successfully established. The SF-21AE cells were found to be persistently infected with this entomopathogen and the healthy cells were supplemented at a period of about one month according to the infection rate. With this cultivation system, the life cycle of this entomopathogen achieved within 2 weeks and the infective merozoites which were capable of proceeding to further infection were released persistently from infected cells. Using histochemical, technique, indirect immunofluorescent assay and electron microscopy, several developing stages could be distinguished. The results of the present in vitro cultivation system could provide an available tool for studding microsporidian and spores free from other entomopathogen contamination.

21-057

INFECTION OF *HELICOVERPA ZEA* (LEPIDOPTERA : NOCTUIDAE) LARVAE AND CULTURED CELLS WITH "FEW-COILED SPORES" OF *NOSEMA FURNACALIS* (MICROSPORA : NOSEMATIDAE)H. Iwano, T. J. Kurti¹ and U. G. Munderloh¹

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Our previous studies showed the population of *Nosema furnacalis* spores was consisted of "few-coiled spores" only, when harvested from cultured cells of *Helicoverpa zea* subcultured eighty times after inoculation of "many-coiled spores" of *N. furnacalis*. We report here those "few-coiled spores" can infect *H. zea* larvae, when injected subcutaneously without priming. Haemocytes were found to harbor developing stages of *N. furnacalis* one day after injection and many spores in various tissues 14 days after injection. Also, "few-coiled spores" could infect *H. zea* cultured cells without priming after the storage for one to three months at 5 °C.

21-059

PCR DETECTION OF MULBERRY DWARF DISEASE-PHYTOPLASMAS IN AN INSECT VECTOR, *HISHIMONAS SELLATUS* AND PHLOEM SAP COLLECTED BY LASER STYLECTOMY

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PCR (polymerase chain reaction) detection of mulberry dwarf disease-phytoplasmas (former mycoplasma-like organisms) in a rhombic-marked leafhopper, *Hishimonus sellatus* and phloem sap was investigated. Two sets of primer pair were used to amplify the target DNA fragment of 16S rRNA genes of phytoplasmas. The preparation of template DNA samples from insects was performed by CTAB method. PCR detection of phytoplasmas was possible in DNA samples prepared from a whole body of infected leafhopper but not in any samples from non-infected insects. The predicted band was also observed in samples prepared from a head, an abdomen and a pair of salivary gland of infected insects, respectively. Pure phloem sap was collected from diseased mulberry by laser stylectomy using *Ricania japonioca* and it was used directly as the DNA template. The predicted band was observed for phloem sap samples collected from diseased plants, but not for any samples collected from healthy plants. PCR products were shown to be the targeted DNA originated from phytoplasmas by analysis of their restriction enzyme digestion or nested PCR. Thus, PCR was very specific and sensitive to detect mulberry dwarf disease-phytoplasmas in the insect vector and phloem sap.

21-060

EFFECT OF COCCIDIA *Adelina* SP. (PROTOZOA: EUCCIDIA) ON TWO LARVAE CETONIINAE: *Cetonia aurata* (LINNE) AND *Oxythyrea funesta* (PODA)

By

A. KHARAZI-PAKDEL & P. ROBERT

An adelina (Protozoa:Eucocciida) strain isolated from *Cetonia aurata* Provoked a lowered fecundity and fertility in infected *C. aurata* and *Oxythyrea funesta* (Col:Scarabaeidae, Cetoniinae) females as compared to untreated females.

Transmission from host to host apparently occurs only Per os.

21-062

MICROBIOLOGICAL CONTROL OF *LYMANTRIA DISPAR* L. WITH *BACILLUS THURINGIENSIS KURSTAKI* IN SARDINIA SUPPORTED BY ECOLOGICAL STUDY

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Observations on the gypsy moth population dynamics have been carried out for over 30 years in Sardinian cork-oak forests. The defoliator fluctuation amplitudes varies from 11 to 5-6 years in different parts of the Island, probably influenced by the climatic conditions and by the composition of the plant communities in the environments studied. Several parameters characterizing each gradation phase of gypsy moth (egg population density, eggs/egg-cluster, fertile egg percentage and parasitism rates) have been obtained; they enable its population trends to be predicted and prove useful in programming pest control operations. In response to the ever-increasing awareness of the need to control the gypsy moth infestations, large cork-oak forest areas have been subjected to microbiological control tests, avoiding any upset to the delicate ecosystemic equilibrium. In particular, various preparations based on *Bacillus thuringiensis kurstaki* (Btk) were distributed aerially, to test their efficacy and the most suitable application modality to a Mediterranean environment. Accurate timing is essential: in the case of low infestations (2 larvae/branch tip), when at least 90% of the larval population consists of I and II instars, 32 BIU/ha determined more than 80% mortality. Application at 60 BIU/ha guaranteed effective control against the highest infestations (5 larvae/branch tip) causing more than 80% mortality. An important advantage is obtained from the application of the undiluted product at ULV, thereby providing aircraft productivity and reducing ground support requirements. In these experiments, *B. thuringiensis* did not prove to have any direct effect on the gypsy moth parasite and predator activity, whose incidence seemed to be dependent on host densities in treated and untreated areas.

21-061

LARGE SCALE USE OF NUCLEAR POLYHEDROSIS VIRUS IN BRAZIL TO CONTROL THE SOYBEAN CATERPILLAR, *ANTICARSIA GEMMATALIS* (LEP.:NOCTUIDAE).

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A nuclear polyhedrosis virus (NPV) of *Anticarsia gemmatalis*, a key soybean defoliator in Brazil, has been used as a microbial insecticide since 1982. Initial use by farmers were based on crude preparations of the NPV, but a wettable powder formulation was developed and made available to the farmers since 1986. Presently, five private companies produce and commercialize the microbial insecticide, and its annual use is estimated at approximately one million hectares of soybean in the country. Analysis of temporal NPV isolates, collected each season after large-scale field applications, indicates that virulence was not significantly affected after more than ten years of virus use as a microbial insecticide. Also, resistance to the NPV could not be detected between *A. gemmatalis* populations collected from different regions in Brazil, even when comparing populations from sites where the NPV had been applied yearly with populations from sites with no history of virus applications. On the other hand, *A. gemmatalis* rapidly developed resistance to the NPV under selection pressure experiments in the laboratory, with resistance ratios reaching over 2,000. The implementation of the programme, the factors related to the success of the NPV as a microbial insecticide, and recent research developments will be discussed.

21-063

DIVERSITY OF NUCLEAR POLYHEDRAL VIRUSES ISOLATED FROM VARIOUS *LYMANTRIA DISPAR* L. POPULATIONS

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Nuclear Polyhedral Viruses isolated from *Lymantria dispar* L. as 3rd-4th instar larvae from Romania, West and East Ukraine, Yugoslavia, Kirgizia, USA and Canada have been examined.

To study diversity of baculoviruses the morphological data obtained by electron microscopy have been analysed. The main criteria were: average number of nucleocapsids included in one superpolyvirion (SPVC) and envelope features of SPVC (polyhedrons).

It was shown that NPV isolated from European and Asiatic populations included virions and polyvirions containing one nucleocapsid, three nucleocapsids (q1, q3 and q4). The NPV isolated from America presented only virions (q1), while q3 and q4 were not significant.

The total number of nucleocapsids in SPVC of NPV isolated from *L. dispar* populations in Europe and Asia was roughly 1,215 nucleocapsids, while only 723 in the American populations. These data are also correlated to biological activity, which was about twice higher in Euro-Asiatic populations.

21-064

ENTOMOGENOUS FUNGI IN RESOURCE MANAGEMENT IN SUSTAINABLE AGRICULTURE AND FORESTRY

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Terrestrial entomogenous fungi are acting like efficient natural enemies of various pest insects. Several fungal species were, and still are given high interest because of their potential as "true" biological agents (which implies that inoculum could be artificially produced). Most of fungal entomopathogens, however, are unlikely to be used in this way in the near future. Given this, a strategy in the view of sustainable agriculture and forestry would be concomitantly, to act on any factor likely to favour the establishment and spread of beneficial fungi in insects populations, and to make sure that no cultural practice disadvantage these microorganisms.

Such objectives require intensive studies, which allow to specify, among others: the identity of naturally occurring entomogenous fungi in agroecosystems, forest areas and natural ecosystems, the importance of connections between these ecosystems, the host spectrum of these pathogens, their ecological requirements, their interactions with other microorganisms (especially in the soil), their susceptibility to cultural practices, first of all the use of pesticides.

These various aspects would be discussed on the basis of data originating from temperate and tropical areas as well, and involving various groups of fungi (Zygomycetes: order Entomophthorales; Ascomycetes: genus *Cordyceps*, and Hyphomycetes) and insects (mostly Hemiptera, Lepidoptera, Coleoptera, and Diptera) in.

21-066

BEAUVERICIN PRODUCTION BY ENTOMOPATHOGENIC FUNGI

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The capability to synthesize beauvericin, a well known bioactive cyclohexadepsipeptide with insecticidal properties, was investigated for various isolates of entomopathogenic fungal species, i.e. *Fusarium subglutinans* from *Ostrinia nubilalis* and *Scirpophaga excerptalis*; *Paecilomyces fumosoroseus* from *Eupithecia innotata*, *Carpocapsa pomonella* and *Melolontha melolontha*; *Beauveria bassiana* from *Pyrrhalta luteola*, *Hylotrupes bajulus*, *Costelytra zealandica*, *Ips typographus*, *Laodelphax striatellus*, *Thaumtopoea wilkinsonii*; *B. brongniartii* from *Holotrichia morosa*, *C. zealandica*, *M. melolontha* and *Balaninus nucum*; *B. felina* from *Anaitis efformata*; *B. vermiconia* from volcanic ash soil.

Fungi were cultured for 4 weeks at 25 °C on autoclaved husked rice kernels, a good substrate for beauvericin synthesis, and the chemical analysis was carried out on fungal cultural extracts by high performance thin layer chromatography (HPTLC) and confirmed by high performance liquid chromatography (HPLC). Beauvericin production among isolates of *Beauveria* species was variable, whereas *P. fumosoroseus* and *Fusarium* isolates produced beauvericin at high levels (up to 300 mg/kg dried culture).

The toxicity of the culture extracts toward brine shrimps (*Artemia salina*) was in general related to the occurrence of the beauvericin (LD₅₀ 3.6 µM), except for some *Beauveria* isolates. This finding led us to suspect that other metabolites are involved in *Beauveria* species toxicity.

21-065

CONTROL OF TERMITES USING ENTOMOPATHOGENIC FUNGI, *Beauveria bassiana* and *Metarhizium anisopliae*.

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With the objective of finding biological alternatives to be used in the control of termites in Brazil, since 1989, the Entomology Department at ESALQ-USP has been studying the use of entomopathogenic fungi in microbial control of these insects.

Laboratory and field studies have focused on control of *Comitermes cumulans* (pasture termite) and *Heterotermes tenuis* (sugarcane subterranean termite). The basic methodology used in our studies has the objective of selecting highly pathogenic isolates to be used with several control strategies.

For control of *C. cumulans*, we have selected *B. bassiana* isolate 447 and *M. anisopliae* isolate 865. These isolates were applied in the field as an inundative introduction that caused 95 to 100% control of small to medium nests. For *H. tenuis*, from 142 *B. bassiana* isolates initially tested, 15 were selected for causing greater than 89% mortality, and among these, isolate 634 was selected as the most promising candidate. This isolate is being tested under field conditions. An inoculative introduction using a corrugated cardboard bait trap has been chosen as the application methodology. This bait trap is highly attractive to *H. tenuis*, and numbers of insects attracted reach as high as 14, 000 per unit. This fungal isolate has also been applied in combination with chemical pesticides and insect growth regulator.

21-067

COMPARISON OF THE VIRULENCE OF HYPHAL BODIES AND CONIDIA OF *VERTICILLIUM LECANII* AGAINST *FRANKLINIELLA OCCIDENTALIS* (Thysanoptera: Insecta)

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The nearctic Western Flower Thrips (WFT), *Frankliniella occidentalis* (Thysanoptera), has recently become a serious pest of economic importance in Central Europe. It attacks a wide range of crops and causes damage directly through feeding, and indirectly through the transmission of plant diseases including important viruses, bacteria, and phytopathogenic fungi. The expensive commercial use of chemicals and their effects on the environment have generated an increasing interest in alternative control strategies (Moritz & Trost 1994; Schreiter et al. 1994, 1995). Moreover, most important thrips pests (WFT, *Thrips tabaci*) are difficult to control chemically and insecticide resistance occurs.

The use of *Verticillium lecanii* may help to reduce pest populations and the transmitted diseases. The host-parasite-interaction starts if the conidia or hyphal bodies (blastospores) of the fungus come into contact with the insect surface. After inoculation with either spore type, larvae and adults of the WFT show similar mortality, if immature stages are inoculated shortly after hatching or moulting (first instar larva: 71,4 h ± 8h; second instar larva: 94,7 h ± 8h). Therefore the formulation of hyphal bodies in submers or of fungal spores in emers cultures leads to an usable antagonist. Different strains of the entomopathogen and different WFT-populations show varying mortalities at subspecies level. The surface interactions let assume high or low incompatibility between the parasite and the host cuticle. For this reason the study of the autecology, ultrastructure and behavior repertoire of all ontogenetic stages after fungal infection is important.

21-068

THE EFFECT OF A NON PATHOGENIC ENDOPHYTIC FUNGUS ON THE DEVELOPMENT OF THE DIAMONDBACK MOTH *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: PLUTELLIDAE)

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Endophytic fungi, living within host plant tissue without causing any symptoms visible from outside, are known to occur in almost all higher plants. Although these fungi are abundant under natural conditions, research has mainly focused on endophytes-grass-relationships, and little is known about the interactions between soil-borne, root-colonizing endophytes and herbivorous insects. In our study we used the soil-borne endophyte *Acremonium alternatum* GAMS to test the hypothesis that this unspecialised fungus is able to affect the relationship between the diamondback moth *P. xylostella* and its host plant Brussels sprouts (*Brassica oleracea* var. *gemmifera*). In experiments single larvae were fed in petridishes with leaves from either treated or untreated plants. First instar larvae suffered increased mortality when fed with leaves of previously inoculated plants and developmental time was retarded. Because plant growth, leaf water, nitrogen or sugar content did not differ, we analysed secondary plant compounds which are known to be affected by fungal growth (i.e. amino acids and phytosterols). The mechanisms for altered development of the larvae will be discussed in respect to these plant metabolites.

21-069

PATHOGENICITY OF THE FUNGAL ENTOMOPATHOGEN *BEAUVERIA BASSIANA* AGAINST SUGARCANE STALKBORERS (LEPIDOPTERA: PYRALIDAE)

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Pathogenicity of the fungus *Beauveria bassiana* was assessed against the Mexican Rice Borer (MRB), *Eoreuma loftini* and the sugarcane borer (SCB), *Diatraea saccharalis*. We studied the effects of a commercial formulation of dry conidia of *B. bassiana* (Mycotrol®, Mycotech Corp.) on different stages of the larval stalkborers in the laboratory. Insects were sprayed with aqueous suspensions of spores containing the wetting agent Silwet and/ or Tween at 0.01 % concentration. Each individual assay assessed the effects of wetting agent alone, low, medium and high doses of conidia. LD50 values were calculated at 5 days after treatment. LD50 values for first- and second-instar MRB were 8.9 and 10.7 spores/mm², respectively. LD50 values for first and second-instar SCB were 276.2 and 340.8 spores/mm², respectively. The mean survival time calculated for first- and second-instar MRB was 3.8 and 3.3 days, respectively. For SCB, the mean survival time of first- and second-instars was 4.7 and 4.8 days, respectively. Thus, it seems that the strain of *B. bassiana* used in Mycotrol® is more virulent to MRB than to SCB. Further studies will be conducted on the effectiveness of the fungal entomopathogen on older larval stages of MRB and SCB. Future field studies will be conducted on the effect of *B. bassiana* in susceptible and moderately resistant varieties of sugarcane.

21-070

VIRULENCE OF STRAIN 257 OF *BEAUVERIA BASSIANA* (BALS.) VUILL. TO DIFFERENT HOSTS

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In the report data is given about the virulence of strain 257 of *B. bassiana* (Bals.) Vuill., isolated from caterpillars of *Erannis bajaria* Schiff. to different hosts. The experiments were carried out under laboratory conditions. The insects were divided into three groups: laboratory-test-insects-larvae L2-3 of *Galleria mellonella* L., (Pyralidae: Lepidoptera) and the adult of *Acanthoscelides obtectus* Say, (Bruchidae: Coleoptera); original host - larvae L3, L4 of *Erannis bajaria* Schiff., (Geometridae: Lepidoptera); an insect from an order, different from the one of the host but included in the infectious spectrum of the pathogene - the adult of *Leptinotarsa decemlineata* Say, (Chrysomelidae: Coleoptera). Konidia and blastospores of the strain were used for the treatment. The letal time, calculated by the probit-logarithmic analysis was considered as the virulence of the strain (LT 50, LT70, LT90). It was established that the strain is specific to a certain extend, concerning its relatively higher virulency to the original host.

The larvae of *G. mellonella*, treated with konidia of the pathogene show "resistance", which we think is due to the inability of the konidia to overcome the defence of the host. The blastospores of the strain are more infectious and quickly penetrate into the body of the host. The values of the letal time (LD 90) are in confidential intervals, similar to the one of the original host. The virulence of the strain to the adults of *A. obtectus* is low. The adult of *A. obtectus* is not from the infectious spectrum of the strain 257 of *B. bassiana*.

21-071

SOME ENTOMOGENOUS SPECIES OF *PAECILOMYCES* GENUS ISOLATED IN JAPAN

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Isolation of *Paecilomyces* species from soil samples by using two selective media and the baiting method was attempted. Many isolates of *P. farinosus* and *P. fumosoroseus* were isolated. *P. tenuipes* was not detected by the methods. The baiting method was most effective because isolates were obtained by the method from soil samples which was not detected by two selective media. Both of the species were virulent to larva of peach fruit moth, *Carposina sasakii* (= *C. niponensis*) which was a key pest of Apple, Japanese pear and peach in Japan.

Four isolates of *Paecilomyces* species were isolated from soil samples collected in Oota, Okinawa, Hiroshima and Tokyo by using the baiting method. They were characterized by their cylindrical and large conidia (8-11x2-3 μ m). They were pathogenic to larvae of peach fruit moth. In 1994, pupae of cicada, *Meimura oalifera* infected with a fungus were collected in Ibaraki. Synnemata erected from head of cicada were 3.5-5 cm and powdery near apex due to the conidial mass. These isolates were resemble closely to them from soil samples. Both of isolates isolated from soil and cicada were identified as *P. cicadae*. It was found that this species was also virulent to lepidopteran insect.

In 1995, *P. amoeneroseus* was isolated in Morioka where was located in the northern part of Japan and was found to be pathogenic to lepidopteran and coleopteran insects.

21-072

QUICK AND LASTING CONTROL EFFECT FROM THE APPLICATION OF *VERTICILLIUM LECANII* (ZIMMERMAN) (HYPHOMYCETALES: MONILIACEAE) PLUS SILWET® L-7607N ON *MYZUS PERSICAE* (SULZER) (HEMIPTERA: APHIDIDAE)

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The use of the entomopathogenic fungus, *Verticillium lecanii* (Zimmerman), as a pest control agent, has attracted applied entomologists' attention because of their low human toxicity and environmental friendliness. However, the application of *V. lecanii* is not expected to produce sufficient control of rapidly reproducible pests such as aphids when their density is high, perhaps owing to its slow action: The reproduction of such pests generally exceeds the outbreak of the disease. In order to improve such a situation, we examined the use of surfactants that exhibit quick aphicidity¹ together with *V. lecanii*. The germination of the blastospores was little suppressed when two 0.1% silicone surfactants were applied: The germination rates were 83.2% and 95.2% in Silwet® L-7607N and L-77 solutions, respectively. The combined application of Silwet® L-7607N and the blastospores on *Myzus persicae* (Sulzer) well met our expectations: the spray of the suspension mixture that contained the blastospores (1.0×10^7 CFU/mL), Silwet® L-7607N (0.1%), and sodium carboxymethyl cellulose (0.1%) over approximately 8-leaf-stage *Nicotiana tabacum* (L.) produced immediate and lasting control effect at 18.9°C and 95.8% RH in averages, whereas the spray of the suspension without the surfactant did not show any aphicidity within 3 days after treatment under the same condition. These results suggest that the application of a silicone surfactant together with *V. lecanii* would produce sufficient control on aphids regardless of their initial density.

1: Imai et al. (1994) *Appl. Entomol. Zool.* 29: 389-393

21-074

DISEASES OF GREYBACK CANEGRUB (COLEOPTERA: SCARABAEIDAE) IN SUGARCANE IN AUSTRALIA

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The melolonthine scarab *Dermolepida albohirtum* (Waterhouse) is the most damaging of the insect pests of sugarcane in Australia. The root-feeding larvae, known as greyback cane-grubs, are difficult to control because they develop in well-grown cane crops which are inaccessible to conventional pesticide application machinery.

Disease incidence was determined during a study of the population dynamics of greyback cane-grub in North Queensland, Australia. The fungus, *Metarhizium anisopliae*, accounted for 31% of cane-grubs which died across all sites. The protozoan *Adelina* sp. had its highest incidence (100%) in cane-grubs which died from Stone River near Ingham in 1994. Seventy-four percent of grubs collected from this site died in the laboratory. Cane-grub population density declined by approximately 80% at Stone River during the 1994 season. Localities with low incidence of *Adelina* in cane-grubs showed little change in population density throughout the season. *Adelina* sp. was also recorded from adult greyback cane-grub.

Other diseases recorded from field-collected cane-grubs included an entomopox virus, bacteria (*Bacillus popilliae* and *Bacillus* nr. *sphaericus*), and a microsporidian resembling *Nosema*. "*Nosema*" sp. was experimentally transmitted to healthy cane-grubs, causing retarded growth and eventual death.

We postulate that the cyclical pattern of outbreaks of greyback cane-grub recorded every 10 - 15 years throughout North Queensland may be caused by variations in the incidence of entomopathogenic diseases. *Adelina* infection, in particular, may influence population dynamics of greyback cane-grub.

21-073

INTERACTIONS BETWEEN A NON-PATHOGENIC FUNGUS, *ASPERGILLUS NIDULANS*, AN ENTOMOPATHOGENIC FUNGUS, *BEAUVERIA BASSIANA* AND THE INSECTS *DYSDERCUS PERUVIANUS* (HEMIPTERA: PYRRHOCORIDAE) AND *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA), RESPECTIVELY.

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The insects were inoculated with the conidia-containing solutions, in order to study their immune response and to investigate possibilities of biological control.

D. peruvianus were inoculated with a Hamilton syringe, in volumes of 2-4 µl (3.2×10^7 conidia/ml). The insects were kept for five days after treatment. The mortality was recorded. Hemolymph was tapped from the hind leg, mixed with saline and plated on selective media. A reduction of the number of the conidia was noted (60-80%). Classic genetic experiments were performed with the survivors of the UT 196 strain in crosses to normal strain. The genotypical analyses indicate that inside the insect occurred several changes in the genome of the fungus affecting their genes expression and meiotic pattern. This was also evidenced by treating conidia with demethylation agent such as 5-azacytidine.

The mortality was not elevated for *A. nidulans* while the tested isolate of *B. bassiana* was highly pathogenic.

Both, *in vitro* and *in vivo* tests with *S. frugiperda*, gave similar results.

*Support CNPq and FAPESP.

21-075

SYNERGISTIC EFFECT OF AN ENTOMOPATHOGENIC FUNGUS (*METARHIZIUM ANISOPLIAE*) WITH CHEMICAL INSECTISIDES AGAINST SCARAB LARVAE.

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An entomopathogenic fungus *M. anisopliae* had not showned the sufficient control effect against soil inhabiting insects such as larvae of scarab beetles by using this fungus singly. In this experiment, we investigated the synergistic action of *M. anisopliae* with several chemical insecticides against scarab larvae.

In the laboratory test, higher mortality effect was obtained by treating conidial suspension of *M. anisopliae* with few doses of insecticides against scarab larvae. In these application, we observed that the larval dead bodies were almost infected with *M. anisopliae*. Hence, it is assumed that the use of *M. anisopliae* with chemical insecticides against scarab larvae will be much effective and take on a important role in the system of IPM.

21-076

SPRAYABLE GRANULE FORMULATIONS FOR *BACILLUS THURINGIENSIS*P. Tamez-Guerra¹, M.R. McGuire², H. Medrano-Roldan³, L.J. Galan-Wong¹, F.E. Vega².¹Departamento de Microbiología e Inmunología, Universidad Autónoma de Nuevo León, Monterrey, Mexico; ²USDA-ARS, Bioactive Agents Research Unit, Peoria, IL, USA; ³Instituto Tecnológico de Durango, Durango, Mexico

Formulations incorporating modified starches and flours were developed for extending the residual activity of *Bacillus thuringiensis* (Bt). The basic formulation contained nixtamalized flour from corn (commonly used in Mexico for industrial purposes), cornstarch, corn oil, lactic acid, alcohol, and sugar. When mixed with water and active ingredient and spray dried, small granules were formed. These granules could then be added to a spray tank and sprayed onto a target. Variations of these ingredients included substituting the nixtamalized flour with pregelatinized flours or starches commonly available in the U.S. Tests were conducted in the laboratory to determine insecticidal activity of Bt and resistance to simulated environmental factors. In diet incorporation tests against three insect species, formulated Bt killed as many or more larvae than unformulated Bt suggesting that the ingredients and processing did not harm the activity. In many cases, activity was increased perhaps due to the feeding stimulant property of the formulation. Cotton leaf surfaces were treated with the formulations and exposed to full simulated sunlight delivered by a Suntest CPS (Heraeus) for 8 h. Unformulated Bt routinely lost about 50% activity during this exposure; however, Bt formulated in nixtamalized flour retained 90-95% activity. There was a reduction of solar stability as the amount of Bt increased in the formulation. We believe these formulations offer a viable, microencapsulated system utilizing surplus, natural materials. Funded in part by CONACYT ref. 3559-N9311.

21-078

DEVELOPMENT OF FOUR CELL LINES FROM THE COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY) (COLEOPTERA: CHRYSOMELIDAE), THEIR CHARACTERIZATION, AND THEIR USE FOR STUDY AND DOSAGE OF ENTOMOPATHOGENS.G. Charpentier¹, S. Bellonci², L. Tian¹.¹ Département de Chimie-biologie, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec, Canada, G9A 5H7. ² Centre de Recherche en Virologie, Institut Armand-Frappier, C.P. 100, Laval-des-Rapides, Québec, Canada, H7V 1B7

Colorado potato beetle is an economically important pest. In order to isolate new pathogens (viruses, microsporidia, etc...) or to evaluate the potency of some pathogens (serovarieties and mutants of *Bacillus thuringiensis*, fungi, etc...) for their control, we established four cell lines from Colorado potato beetle. One cell line was initiated in the M3 medium supplemented with 20% fetal bovine serum (FBS) from embryonated eggs collected from field or in our laboratory colony. It has been transferred after some passages in Ex-Cell 400 medium + 20% FBS. One other cell line was started from hemocytes of larvae in Ex-Cell 400 + 5% FBS. Finally, two other cell lines were started from hemocytes of adults; one in Ex-Cell 400 + 20% FBS and 1% of lipid mixture and the other with 5% FBS only. These cell lines have been characterized by their morphology in light and electron microscopy, their karyotypes and measurement of cell growth. Isozyme analysis are now in progress. Each cell line differed in morphological, karyotypical and growth patterns. The cell line started from embryonated eggs was growing slower than the others. Cytotoxicity of solubilized crystal delta-endotoxins from different *Bacillus thuringiensis* formulations (M-one, Trident, MYX-1806, Teknar HPD, and Thuricide) was tested on these cell lines. Our Colorado potato beetle cell lines are sensitive to the solubilized toxins of some strains of *B. thuringiensis* (serovarieties *San Diego* and *tenebrionis*). We also tested the cytotoxicity of destruxins from *Metarhizium anisopliae* (an entomopathogenic fungi) on these cell lines. We are now evaluating the permissivity of these cell lines for some entomoviruses.

21-077

COMPUTERIZED ECOLOGICAL DATABASE OF THE WORLD'S INSECT PATHOGENS

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There is a need for insect pathologists, ecologists, and government regulators to better understand the ecology of pathogens and their hosts. Can we predict the host-specificity or host range of a pathogen? This database can help regulators and decision makers involved in biological control assess the risk of introduced pathogens harming beneficial and endangered insect species. The database can also be used to find out what species have been observed infecting pests.

The types of information entered into the insect pathogen database include: insect species, family, and order; pathogen species, two higher taxa, and group (i.e. bacteria, fungus, microsporidia, protozoa, virus); life stage of insect susceptible to infection; host tissue infected; intermediate host; habitat of insect host; plants or animals on which the insect host feeds; number of host generations per year; whether the insect/pathogen association was observed in the field or the laboratory; the country in which the insect/pathogen association was observed; the name(s) of the observer or the reference in which the insect/pathogen association was cited.

The database has over 4,000 associations between species of pathogen and species of insect, mite or tick. In 1996 it will be accessible on the World Wide Web.

21-079

THE BACTERICIDAL EFFECT OF ANTIBACTERIAL PEPTIDE FROM CHINESE OAK SILKWORM TO THE PATHOGEN OF BACTERIAL ULCER AND YELLOW SHOOT DISEASES IN CITRUS

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In this article, the bactericidal effect of antibacterial peptide (AP) from Chinese oak silkworm pupae, *Antheraea pernyi* on the growth of the pathogens of bacterial ulcer (*Xanthomonas campestris* PV *citri*) and yellow shoot disease (Bacterium like Organism, BLO) was firstly reported.

The inducible AP was immunized from the haemolymph of Chinese oak silkworm pupae by injecting with *E. coli* K12D31. The AP was purified by boiling (100°C, 30min), centrifuging and separating with Sephadex G-50. The purified AP was labelled with fluorescein yellow.

Both pathogens were treated with 10µg/ml of AP. The optimal effective time was 20-60 min for killing the bacteria. Observation under the laser scanning confocal microscopy and electron scanning microscopy showed that the primary targets of AP were the cell membrane. In the initial action stage, the receptor sites were seriously injured and some small channels were found on the cell surface, then the intracellular substances were pressed out of the cell, at last the cells became empty and died.

21-080

MICRO AND MACRO ORGANISMS OF WILD APIDAE ADULTS IN SOUTHERN ITALY.

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The research carried out in the Apulian Region (Southern Italy) during 1988-1995 pointed out the presence of micro and macro organisms in *Bombus* spp., *Psithyrus* spp., *Xylocopa violacea* L., *Andrena carbonaria* L., *Halictus brunnescens* Eversmann and *H. fulvipes* Klug adults. The following Protozoa were isolated in *Bombus* and *Psithyrus*:

-*Apicystis* (= *Mattesia*) *bombi* (Liu, Macfarlane & Pengelly), reported for the first time in Italy in 1988, was more frequently found in *Bombus terrestris* L. during July 1995 (32%), alone or associated with *Nosema bombi* and *Critidia bombi*.

-*Nosema bombi* Fantham et Porter, constantly isolated from *B. terrestris*, *Megabombus pascuorum* Scopoli, *Psithyrus vestalis* Geoffr., was more frequent in anthropized biotopes and experimental fields.

-*Critidia bombi* sp. n. was primarily found in the gut of *B. terrestris*, *M. pascuorum*, *Megabombus hortorum* L., *Megabombus ruderatus* Fab., *Pyrobombus sicheli* Rad. Heavily infected adults had problems in flying.

The Nematode *Sphaerularia bombi* Dufour was isolated from *B. terrestris*, *M. pascuorum*, *P. sicheli* and *M. ruderatus* queens that were parasitized up to 80%. Juvenile stages of *S. bombi* were also found in two workers of *P. sicheli* in May 1994 and in two workers of *B. terrestris* in April 1995.

Up to four queens of *B. terrestris* proved to be parasitized by *Syntrenus* sp. (Hymenoptera: Braconidae) during May 1990-93 and 1995. A percentage ranging between 3.0% and 75% of males, workers and seldom queens of *Bombus* was parasitized by the Diptera *Conops flavipes* De G. Different species of Protozoa, not yet identified, were found in other Apidae during 1994-95. A *Critidia* sp., in only a male out of 7 collected in March 1994, and a Gregarine *Leydyana* sp., in one sample collected in September 1994 in the Pollino National Park, were found in *Xylocopa violacea*. Different species of Gregarines were isolated from *Andrena carbonaria*, *Halictus brunnescens* and *H. fulvipes*. In both species of *Halictus* the Gregarines were present in 90%-95% of the specimens during September 1995.

21-082

Observations on *Bacillus thuringiensis* toxicity against *Ceratitis capitata* Wied. (Dipt. Tephritidae)

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The medfly (*Ceratitis capitata* Wied.) is considered of great economic importance in the Mediterranean area, since it attacks a variety of fruits. Control of this pest is based on cover sprays with organophosphorous insecticides or on bait sprays with protein hydrolyzate, that have detrimental effects on beneficial entomofauna and cause environmental problems. The development of a safe biocontrol agent to replace organophosphorous insecticides in bait sprays technique would solve these problems.

For this purpose, we have obtained from about 100 samples of soil, collected in two Mediterranean islands (Sardinia and Corsica) and in Africa (Angola and Zimbabwe), 70 isolates of Bt with typical parasporal crystal inclusions of different sizes and shapes.

Preliminary bioassays of Bt against a laboratory population of larvae and adults of *C. capitata*, showed a high variability of toxicity correlated to the isolates and to the insect life stage. The larvae were more susceptible than adults to the same Bt suspension and the effect was dose-dependent. At very low doses the development of the larvae was delayed. In some case, with a dose of 1.3×10^9 spores and crystals per gram of larval diet, 100% of mortality was reached.

The adult fly showed a remarkable higher level of resistance compared to the larvae, reaching a maximum peak of mortality of 40%.

Some isolates of Bt were active only on larvae or on adults.

21-081

EFFECT OF STRESS ON THE SUSCEPTIBILITY OF INSECTS TO PATHOGENS

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Especially in relation to insect cultures, it is frequently stated that stress increases susceptibility of insects to diseases. However, a search of the literature gave little support for such a general statement. Two pest insects, the rose-grain aphid *Metopolophium dirhodum* and the diamondback moth *Plutella xylostella*, were therefore subjected to various stress treatments.

Rose-grain aphids were sprayed with sublethal concentrations of the pyrethroid insecticide cypermethrin or reared on a resistant wheat cultivar before being infected with the hyphomycete fungus *Verticillium lecanii*. None of these two stress treatments resulted in a consistently higher mortality of stressed aphids from fungus compared to nonstressed aphids.

Stress caused by cypermethrin or by the botanical insecticide azadirachtin also failed to increase the susceptibility of larvae of the diamondback moth to the bacterial pathogen *Bacillus thuringiensis*. On the other hand, some resistant host plants were found to increase *B. thuringiensis*-related mortality in *P. xylostella*.

It has to be concluded that stress as a general phenomenon does not necessarily increase the susceptibility of insects to diseases but that individual stress factors, such as host plant resistance, can have an effect and should be considered in integrated pest management systems.

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21-083

PATHOGENICITY OF *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* AGAINST *SPILOSOMA OBLIQUA* (W)

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Jute hairy caterpillar *Spilosoma obliqua* (W), a serious pest of jute crop, also attacks a number of other crops such as cotton, groundnut, brinjal, cabbage, cauliflower, soybean, mashkalai etc. To overcome the adverse effects of synthetic pesticides in agroecosystem, use of microbial pathogens particularly bacterial pathogen can play a significant role in this regard. Because bacterial pathogens are safe, selective and compatible with most pest control options of integrated pest management. The pathogenicity (LC₅₀, LC₉₀, LT₅₀, LT₉₀ values) of the bacterial pathogen, *Bacillus thuringiensis* var. *kurstaki* was determined against different instars of *S. obliqua*. The effect of the pathogen on the growth and development of the insect pest was also studied. *B. thuringiensis* could be an alternative of synthetic pesticides for the management of *S. obliqua*.

21-084

EXPRESSION OF *BACILLUS THURINGIENSIS* INSECTICIDAL CRYSTAL PROTEIN FRAGMENTS ENGINEERED INTO COMMERCIAL WALNUTS (*JUGLANS REGIA* L.)

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In 1989 studies began to incorporate the genes responsible for the production of *Bacillus thuringiensis* insecticidal crystal protein fragments (ICPFs) into the genome of walnut. If successful, the research could have impact on both production and postharvest insect control methods. Initial studies with the purified ICPFs were conducted to determine the susceptibility of three important production/storage pests, navel orangeworm (*Amyelois transitella*), codling moth (*Cydia pomonella*), and Indianmeal moth (*Plodia interpunctella*) of commercial walnuts to two ICPFs (CryIA(b) and CryIA(c)). Both proteins were toxic to the insects. *A. transitella* was the least and *P. interpunctella* the most susceptible species. ICPFs slowed *A. transitella* development but few other sublethal effects were noted. Transgenic walnut embryos known to contain ICPF genes based on initial screenings by GUS, kanamycin resistance and/or southern blot were fed to *C. pomonella* or *A. transitella* larvae. If significant mortality could be obtained with these species toxicity to *P. interpunctella* would likely be assured. Early presumptive engineered embryos proved to have little activity towards the subject pests indicating expression problems. With later constructs we have obtained a number of embryo lines expressing varying levels of ICPFs. The embryos screened had low, medium, and high levels of expression. Feeding on high expression embryos was minimal. Walnut seedlings from embryos with high levels of expression were planted in 1995.

21-086

DIFFERENCE OF ANTIBACTERIAL ACTIVITY INDUCIBLE IN THE HEMOLYMPH AMONG THE SILKWORM, *BOMBYX MORI*, RACES BY IMMUNIZATION (LEPIDOPTERA:BOMBYCIDAE)

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To know a concern of antibacterial activity with differences of healthiness among the silkworm races, antibacterial assay by measuring of bacterial growth inhibition was examined. The respective hemolymph were collected from 10 races of silkworm (4 of chinese, 3 of japanese, 2 of european strains and 1 of hybrid) with injection of dead bacteria and were used.

These activities were found to differ respectively. DAIZO (a chinese strain)'s activity was especially higher than the others while its overall hemolymph proteins concentration was the lowest.

21-085

ANTAGONISTIC EFFECT OF Cry1Ac AND Cyt1A TOXINS OF *Bacillus thuringiensis* ON *Trichoplusia ni* (Hübner) (LEPIDOPTERA: NOCTUIDAE) LARVAE AND TN5B1-4 CELLS.

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Most of the *Bacillus thuringiensis* (*Bt*) strains show a set of different protoxins in their parasporal crystals. Some of these combinations clearly interact synergistically, like the toxins present in *Bt israelensis*. Synergism in other combinations is only speculative. This report introduces a novel joint action between toxins from significantly different strains. A series of *per os* bioassays against late 1st instar larvae of the cabbage looper, *Trichoplusia ni*, was conducted in order to estimate LC₅₀s of pure Cry1Ac crystals from *Bt kurstaki*, pure Cyt1A crystals from *Bt israelensis*, and two different combinations of both toxins. The first LC₅₀ was estimated at 2.46 ng/cm² diet, while Cyt1A crystals showed no toxicity, even at very high doses. LC₅₀s were estimated at 15.69 and 19.05 ng Cry1Ac crystals per cm² diet, when mixed with 100 and 1,000 ng Cyt1A crystals per cm² diet, respectively. *In vitro* bioassays of pure, trypsin activated toxins against Tn5B1-4 cells of *T. ni*, showed contrasting susceptibility. LC₅₀s were estimated at 4,967 and 11.69 ng/ml medium of Cry1Ac and Cyt1A crystals, respectively. When mixtures of toxins were assayed using different proportions, eight different LC₅₀s were estimated. All of them were significantly higher when compared to the expected LC₅₀s of the mixtures. This results indicate an obvious antagonism between toxins, both, *in vivo* and *in vitro*. These are the first evidences of a novel joint effect of *Bt* toxins from different strains. Various possible explanations on this relationship are discussed.

21-087

EFFECT OF *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* δ -ENDOTOXIN ON INSECT FAT BODY STRUCTURE

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The effect of *Bacillus thuringiensis* var. *kurstaki* HD-1 δ -endotoxin was determined on the structure and function in isolated fat body tissue from fall webworm, *Hyphantria cunea* Drury. Protein synthesis and toxin-binding experiment were performed by autoradiography, immunocytochemistry, and scanning electron microscopy.

After fat body exposure to 62KDa endotoxin over 150 μ g/ml, protein synthesis was completely inhibited. Immunostaining was observed 30min after *in vitro* fat body exposure to toxin. By 1h after exposure, high degree of staining is observed along the fat body membrane, resulting in separation of basal lamina from fat body and cell disruption. After 2h cellular damage had increased, with protrusion of cytoplasmic contents as well as cell decay.

21-088

EFFECT OF *Bacillus thuringiensis* and INSECT GROWTH REGULATOR ON THE CONTROL OF THE DIAMOND BACK MOTH, *Plutella xylostella* (L., 1758) (LEPIDOPTERA: PLUTELLIDAE), IN CABBAGE.

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The control of *Plutella xylostella* in cabbage using conventional insecticides is a serious problem all over the world. The purpose of the experiment was to be study the effect of *Bacillus thuringiensis* var. Aisawai (AGREE WP) and insect growth regulator (Chlorfluazuron 5% EC) as alternative of control of *P. xylostella*, under field conditions, in São Paulo State/Brazil, during 1993. The defoliation and the quantity of marketable standard cabbage head were evaluated, after 5 weekly applications of *B. thuringiensis* (rates: 750, 1000 and 1500 g c.p./ha), Chlorfluazuron 5% EC (rates: 400 and 600 ml c.p./ha) and Permethrin 38,4% EC (rate: 696 ml c.p./ha). The treatments were distributed in random blocks, with 4 replications. Each plot consisted of 40 plants. The cultivar utilized was Matsukaze.

The results showed that *B. thuringiensis* (rates: 750, 1000 and 1500 g c.p./ha), Chlorfluazuron 5% EC (rates: 400 and 600 ml c.p./ha) obtained 88, 94, 100, 95 and 96% of marketable standard cabbage head, respectively, showing that treatments may be used in Integrated pest management programs. The treatment with pyrethroid insecticide, Permethrin 35,4% EC (rate: 696 ml c.p./ha), obtained just 12% of marketable standard cabbage head. The control treatment (without applications) showed severe attack by *P. xylostella*, whereas all the cabbage head produced were below the market standard.

21-090

ENTOMOPATHOGENIC FUNGI DEGRADE EPICUTICULAR HYDROCARBONS OF *TRIATOMA INFESTANS* (HEMIPTERA: REDUVIIDAE)

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The role of insect cuticular hydrocarbons as a barrier against water loss, agrochemical and toxic penetration has been frequently addressed.

Because nearly all fungal infections are produced by invasion through the host's cuticle, this organ is also the primary defense against microbial diseases.

In this paper we analyze the ability of entomopathogenic fungi to utilize insect epicuticular hydrocarbons. Strains of *B. bassiana* and *M. anisopliae* were grown on synthetic hydrocarbon enriched media as the only carbon source. Similar experiments were performed with hydrocarbons or non-hydrocarbon lipid fractions extracted from the blood-sucking insect *T. infestans*.

Conidia production was significantly higher in media containing cuticular hydrocarbons rather than other cuticular lipid fractions or synthetic hydrocarbons.

Fungal hydrocarbon composition is modified when grown in a hydrocarbon enriched media as shown by gas-chromatography analyses.

Hydrocarbon consumption was measured using synthetic radioactive hydrocarbon. The degradation products were free fatty acids, polar lipids and acylglycerols as shown by radio-thin layer chromatography and radio-high pressure liquid chromatography analyses.

21-089

BINDING OF *BACILLUS THURINGIENSIS* INSECTICIDAL CRYSTAL PROTEINS TO THE MIDGUT OF *HELICOVERPA ARMIGERA* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE)

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A key step in the toxic action of *Bacillus thuringiensis* insecticidal crystal proteins (ICP) is the binding of the activated toxin to the midgut epithelium. Changes in the affinity and/or concentration of the binding site have been identified as a basis for resistance to Bt.

As part of a project on resistance to CryIAC, the ICP produced in transgenic cotton, by *Helicoverpa armigera* we determined the toxicity of various ICPs for *H. armigera* and found that there were only a few ICPs that were sufficiently toxic to be useful alternatives to CryIAC in resistance management. Relative affinity of the *H. armigera*-toxic ICPs and CryIAC for a midgut binding site was determined by competitive binding assays.

A CryIAC binding protein was purified from *H. armigera* midgut epithelium on a CryIAC affinity column and characterised.

21-091

TACTICS OF THE MICROBIOLOGICAL PROTECTION OF VINEYARDS FROM LEPIDOPTERA PEST INSECTS

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Among the Lepidoptera insects the grape berry moth, *Lobesia botrana* Den. & Schiff. (Tortricidae) is one of the important pest of vineyards in Georgia. On the early grape vine sort - "Tbilisuri" - this pest has been developed in two generations. The first damages flower cluster, second - the ripe berries. The scheme of the microbiological protection of "Tbilisuri" has been developed: one treatment before the completing of flowering and two - during the riping of grape. The same pest on the production grape vine sorts developed in three generations, where the larvae of second and third generations are more harmful. The bacterial preparations on the base of *Bacillus thuringiensis* are used both early and industry grape vine sorts from the grape berry moth and other species of *Lepidoptera* insects in the main regions of viticulture of Georgia.

21-092

MANAGEMENT OF DIAMONDBACK MOTH, *PLUTELLA* *XYLOSTELLA*, USING BIOLOGICAL INSECTICIDES

Abstract. Several studies were conducted to control diamondback moth (DBM), *Plutella xylostella* (L.), on cabbage (*Brassicae oleracea* var. *capitata* L.) using conventional, biological and botanical insecticides in Tropical Research and Education Center, Homestead, Florida, USA. Emamectin benzoate alone or in rotation with Dipel 2x (1.0 lb./A) or Xentari (1.0 lb./A) significantly reduced *P. xylostella* populations. In the second test, all formulations of *B. thuringiensis* (ABG 6347, Dipel 2x, San 415 & 420, Agree, Able, & Agree in combination with Able) significantly reduced *P. xylostella* populations. In the third study, significantly lowest number of *P. xylostella* larvae was recorded on Xentari treated plants. Other *B. thuringiensis* formulations (Crymax, Agree Dipel 2X, LoCh, & Biobit) also reduced *P. xylostella* larvae when compared with the nontreated control. In the fourth test, *Anagrapha falcifera* (AfNPV) virus performance was comparable with different *B. thuringiensis* formulations (Able, Agree in combination with Able, Xentari, Crymax, & Biobit HP) in controlling diamondback moth. Neemix and Regent also significantly reduced this pest. In the fifth test, different formulations of Azadirachtin significantly increased marketability of cabbage by controlling *P. xylostella* larvae. This information is useful to manage diamondback moth populations in commercial fields using various insecticides in rotation.

21-094

ACTIVITY OF SOME STRAINS OF *BACILLUS THURINGIENSIS* AGAINST CORN BORERS IN EGYPT

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Studies have been carried out on the effect of the microbial control agent *Bacillus thuringiensis* on the two lepidopterous corn borers *Chilo agamemnon* and *Ostrinia nubilalis* which are considered as major agricultural pests in Egypt. The potential activity of some strains of *B. thuringiensis* was evaluated against the second instar larvae of the two corn borer species. Data on the LC₅₀, slopes, 95% confidence limits and potencies of the tested varieties were determined. With respect to *O. nubilalis*, the data indicate that Dipel 2X (*Bacillus thuringiensis* var. *kurstaki* HD-1) is the most potent. The LC₅₀ for Dipel 2X was 2.68 ug/gm diet. The LC₅₀ for other strains ranged between 3.12 and 31.95 ug/gm. In the case of *C. agamemnon*, the lowest LC₅₀ was also recorded with Dipel 2X being 3.68 ug/gm diet. Concerning other tested strains, the LC₅₀ ranged between 6.21 - 15.11 ug/gm. The results generally show that Dipel 2X (*B. thuringiensis* var. *kurstaki* HD-1) is the most potent against the two tested species, as compared to the standard.

21-093

NATURALIS™: A BIOPESTICIDE (*BEAUVERIA BASSIANA* JW-1) FOR CONTROL OF ECONOMIC INSECTS IN FIELD CROPS, VEGETABLES, ORNAMENTALS, AND GREENHOUSES WITH EMPHASIS ON CONTROL OF *BEMISIA*

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Naturalis™ is a natural biological control product that has activity against major pests of cotton, vegetables, and ornamentals. The active ingredient, an insect specific fungus, is contained in the patented product which is registered by EPA in the United States and other countries. The Greenhouse Growers Association in the U.S. named Naturalis as the number one product for 1996 for use against insects, particularly *Bemisia*, thrips, and aphids. Laboratory and field evaluations have established that Naturalis is highly compatible with natural predators and parasites, i.e., *Chrysoperla* sp., *Eretmocerus* sp., *Encarsia* sp., *Scymus* sp. and others. Results of U.S. and international field trials in field crops, vegetables, ornamentals, and greenhouses will be presented.

21-095

CONTROL OF PLUM CURCULIO ADULTS WITH *STEINERNEMA CARPOCAPSAE*

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The entomopathogenic nematode *Steinernema carpocapsae* All strain was evaluated for field effectiveness against adult plum curculio, *Conotrachelus nemuphar* Herbst, a key apple pest in Quebec apple orchards. In a first experiment, the persistence of *S. carpocapsae* on leaves, flower clusters and twigs was monitored using bioassays with *Galleria mellonella* larvae. In 1992, 1993, 1994 and 1995, *S. carpocapsae* juveniles remained infective on apple leaves 38, 42, 98 and 24 h after application respectively. In bioassays, the percentage of *G. mellonella* mortality was consistently higher on leaves, intermediate on flower clusters and lower on twigs for all application dates. After a 12 and 24 h exposure period, the survival rate of *S. carpocapsae* on leaves was estimated at 35 and 15% in 1994 and at 87 and 0% in 1995 respectively. In a second experiment in 1993 and 1994, three consecutive border row sprays of *S. carpocapsae* were applied with a hand gun sprayer to the foliage and the ground of an insecticide-free orchard against *C. nemuphar* adults. Plum curculio damage at harvest reached 5% the first year and 55% the second year, as compared to 80 and 85% in an adjacent insecticide-free orchard, and 0.0 and 0.5% in another adjacent orchard protected with chemical insecticides. In a third experiment performed in 1994, multiple broadcast sprays of *S. carpocapsae* applied with an orchard sprayer at 200 PSI showed no significant effectiveness on fruit damage caused by *C. nemuphar*. In a fourth experiment performed in 1993 and 1995, nematode suspensions applied at the base of tree trunks significantly reduced by 82 and 100% respectively the survival of plum curculio adults maintained within this habitat over a 7 day period. *G. mellonella* mortality from bioassays performed on nematode treated soil samples averaged 100, 75 and 40% after 4, 7 and 10 days respectively.

Section 22

Integrated Pest Management

22-001

INTRODUCTION: IPM, APPLYING PRINCIPLES OF COMMUNITY ECOLOGY

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IPM has been successful in reducing the input of pesticides while maintaining production of high quality fruit. Further reductions in pesticide will come in the future with refinements in methodology, but such advances will be incremental. Major advances in IPM have been attained in specific regions with changes in philosophy, such as pest management through manipulating insect behavior. Are other major advances in pesticide reduction possible? In my opinion one way is with application of principles of community ecology to pest management. By manipulating the agroecosystem of the orchard we will be able to create orchards that are less dominated by pests and require less pesticides. This symposium was organized to present several examples of projects based on this philosophy of pest management and to provide a forum for discussion of how this type of ecological pest management can be implemented.

22-002

EVOLVING ECOSYSTEMS APPROACHES TO FRUIT INSECT PEST MANAGEMENT

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Ecological studies of agroecosystems have demonstrated both significant environmental problems associated with the intensive physical and chemical control of highly simplified crop production systems, and the largely untapped opportunities for knowledge intensive bioecological design and management of more complex systems. Barriers for change often overemphasise economics and lack of appropriate knowledge and technology. To address these barriers and to facilitate change, two approaches were proposed.

First, an overlapping three stage- efficiency, substitution, redesign (ESR)- evolutionary approach has been developed, together with a set of strategic questions for growers and support personnel.

Second, results of a decade of research on the behavior of the plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae) are presented. Most adults emigrate from apple orchards in the fall and overwinter in surrounding forests.; they re-invade the orchards in spring. We implemented a program whereby only peripheral zones that have more than 1% oviposition scars are treated, resulting in a reduction of ca. 60% of insecticides normally recommended for this pest. Based on sound research and field information, apple growers experienced a shift in risk aversion to achieve a step forward in reducing insecticide use.

22-003

GLOBAL AND REGIONAL PEST INSECTS AND THEIR ANTAGONISTS IN ORCHARDS: SPATIAL DYNAMICS.

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The spatial dynamics of herbivores gains importance when slowly acting pest management tools are employed. The temporal pattern of the techniques to be used in an agroecosystem has received some attention, particularly with respect to the preservation of certain natural enemies (e.g. predatory mites) as well as with respect to resistance management programs. The spatial pattern of the techniques applied in neighbouring plots, however, deserves increased attention especially when slowly acting pest management techniques are involved. This will be exemplified with *Cydia pomonella*. The flight capacity of this moth, dependant on age, sex and mating status, as well as its reproductive behaviour under the influence of sublethal insecticide doses were investigated in the laboratory.

The spatial dynamics of natural enemies are directed by a number of different cues. Knowledge of these cues may contribute to a more efficient biological control of pest species in the future. We investigated the searching behaviour of parasitic antagonists of *Phyllonorycter* spp. The role of herbivore induced volatiles as well as of vibrational stimuli will be discussed.

22-004

ROLE OF HEDGEROWS AND GROUND COVER MANAGEMENT ON ARTHROPOD POPULATIONS IN PEAR ORCHARDS

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With the failure of conventional pest management against the pear psylla *Psylla pyri* (L.) (HOMOPTERA: Psyllidae), strategies have been developed in order to enhance beneficial arthropod numbers (among them *Anthocoris nemoralis* (F.) (HETEROPTERA: Anthocoridae)).

Modifying the pear orchard vegetal environment is one of the integrated pest management (IPM) strategy that increases the agrosystem vegetal diversity allowing thus a better balance between phytophagous arthropods and their natural enemies.

We studied the arthropod fauna sampled from the pear orchard and its surroundings: hedgerow tree species canopies and different ground covers.

Communities were characterized by multivariate analysis: vegetal specificity for arthropod complex, predator-prey associations, subcommunities organization related to phytophagous families.

Inter-relationships between pear tree and hedgerow tree species were studied by Spearman's rank correlations. For instance, both *Fraxinus excelsior* L. and *Hedera helix* L. display a diversified fauna correlated with the pear community, but without significant correlation between each other. Their role towards pear is different: *F. excelsior* hosts specific psylla and cecidomyies, providing food for pear beneficial arthropods while *H. helix* rather acts as a shelter species.

As a ground cover, we tested: 1) bare ground, 2) natural green cover and 3) sown green cover. The structure and numbers of arthropod populations sampled in pear trees were different in these 3 types of management.

These results outline interactions between hedgerow (or ground cover) and pear arthropod populations. Manipulating the vegetal environment by selected species seems a hopeful way for optimizing the pear orchard IPM.

22-006

CHANGES IN THE POPULATION COMPOSITION IN IPM APPLE ORCHARDS UNDER CONTINENTAL CLIMATIC CONDITIONS IN HUNGARY

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Hungary, in the Carpathian Basin has a continental climate and accordingly the fauna are different in many details from the fauna of the West European countries. There are many similarities and many dissimilarities between the phytophagous and zoophagous species occurring in the orchards of Hungary and of West Europe. The biology of pests, their predators and parasitoids may be different, too.

In a comparative experiment the effect of the broad spectrum and selective insecticides was studied on the population composition of arthropod population under this climatic conditions.

Owing to regular use of selective insecticides also some so-called secondary or potential pests different from those of West Europe appeared: San Jose scale, pear tingid (*Stephanitis pyri*), *Peritelus familiaris* (a curculionid beetle). Some pests, e.g. San Jose scale, codling moth have two, sometimes two and a half generations in the Hungarian orchards. These circumstances without doubt modify the develop of IPM system.

22-005

INFLUENCE OF FLOWERING PLANTS AND SELECTIVE INSECTICIDES ON OCCURRENCE OF BENEFICIAL AND HARMFUL ARTHROPODS, DISEASES AND YIELD.

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The observations were conducted on seven plots of apple orchard for four years. On six "integrated" plots only selective or partially selective insecticides (diflubenzuron, teflubenzuron, fenoxycarb, pirimicarb, phosalone) and fenitrothion were used. Also predatory mite *Typhlodromus pyri* (Phytoseiidae) was introduced. On five of those plots four species of flowering plants (wild heliotrope, buckwheat, roquette, mustard) were maintained in order to attract natural enemies of the pests.

The check plot (no 7) was intensively treated with broad spectrum of insecticides. Low differences in population of beneficial arthropods and pest species were visible within particular "integrated" plots. On all-except one-integrated plots the phytophagous mites (*Panonychus ulmi* Koch., *Tetranychus urticae* Koch.) were kept on very low level by *T. pyri* occurring during all four years in high numbers. Contrary, on check plot, *T. pyri* appeared sporadically and phytophagous mites exceeded threshold level. Four groups of beneficial arthropods occurred on "integrated" plots and flowering plants: spiders, predatory bugs lady beetles, parasitic wasps, lacewings and hover flies. Only three pest groups (leaf rollers, bugs and aphids), occurred in relatively high numbers and had to be sprayed. Several others important pest species in Poland (codling moth, apple sawfly, leaf miners, apple blossom weevil) appeared in low numbers and were not controlled at all. The obtained results indicated that using limited number of selective pesticides one can obtain the yield and quality of the apples similar on only slightly lower than yield from check plot sprayed more often with non selective pesticides.

22-007

ECOSYSTEM APPROACHES TO MANAGING INSECT PESTS OF FRUIT

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AREAWIDE CODLING MOTH MATING DISRUPTION AND EFFECTS ON BIOLOGICAL CONTROL

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Codling moth is the key pest of pome fruit production in the western USA. Most insecticides are directed at control of this pest resulting in the disruption of natural controls for many other pests. The control of codling moth through the use of pheromones, mating disruption, became a reality in 1991 with the registration of Isomate-C (Pacific Biocontrol). The potential of mating disruption as a control for codling moth was demonstrated in small orchard plots, 2-5 hectares, using different pheromone dispensing systems between 1992 and 1995. The level of codling moth control in mating disrupted blocks was as good as blocks using conventional control methods when sufficient dispensers were applied per hectare and pest densities were low.

The detection of codling moth populations resistant to organophosphate insecticides, especially in California, and the potential to document possible reversion of resistance prompted the establishment of an areawide management project, Randall Island, in 1993. In 1994 the USDA-ARS funded a codling moth areawide management project which established five sites of 160-500 contiguous hectares in three western states. Reduced use of broad-spectrum insecticides at these sites has resulted in higher levels of natural enemies of pests, such as aphids and pear psylla, and provided an opportunity to evaluate the augmentative release of selected biological control agents. At one site, Oroville, mating disruption has been combined with release of sterile moths to suppress codling moth populations.

22-008

ECOLOGICAL IMPACT OF THREE PEST MANAGEMENT STRATEGIES IN NEW ZEALAND APPLE ORCHARDS

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Three apple pest management regimes are being compared for their ecological impacts, and their ability to ensure the economic production of high quality apples. Species diversity, pest status and fruit quality are being evaluated under i) Conventional, (ii) Integrated, and (iii) Biological fruit production. The Conventional system follows current export orchard practices with broad-spectrum pesticides, while the Integrated system uses a wider range of control methods, including minimal applications, preferably of selective pesticides. The Biological system uses mating disruption for codling moth, "Thuricide" for leafrollers, copper/slaked lime for diseases, and matting or mulches for weed control.

Conventional production has few insects present during the season and little damage at harvest, but increasing insecticide resistance problems and changing markets put the sustainability of this system in doubt. In the Integrated system, excellent control of lepidoptera has been achieved using tebufenozide, with little impact on natural enemies. Further development of the system is needed, but the economics appear favourable for future export. In the biological system, the fungicide sprays impact adversely on some natural enemies. The economic sustainability of this system is dependent at present on the local market price, and for the future on innovative technology to overcome quarantine pests.

22-010

THE INTERNATIONAL AGRICULTURAL RESEARCH CENTRES AND THE DEVELOPMENT OF INSECT-RESISTANT CROPS

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The International Agricultural Research Centres (IARCs) of the Consultative Group on International Agricultural Research (CGIAR) are custodians of the world's largest *ex situ* germplasm collection. Collectively, they conserve about a half million accessions of major food and fodder crops. Active evaluation and use of the collections for host resistance breeding has been a major objective of the CGIAR Centres as a means to ensure sustainable crop productivity increases in an environmentally friendly way. Both collectively and individually, the Centres can provide many successful examples of the effective use of the collections for insect resistance breeding. This paper reviews and analyzes how effective alliances between entomologists, Centre specialists and collaborators in other disciplines can lead to insect-resistant crops. Challenges and opportunities for the further development of insect-resistant crops are examined in the light of concerted efforts on the part of key players in germplasm conservation and use.

22-009

GENETIC RESOURCE CONSERVATION AND PLANT RESISTANCE TO INSECTS

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There has been a steadily increasing output of printed material on the importance of plant biodiversity and genetic resource conservation to world agriculture. This literature, in the form of books and reviews, research articles, newsletters, and journals on genetic resources issues, has stressed the crucial role of genetic resources in the development of new, higher yielding, disease resistant, and better quality crops. As well, the usefulness of conserved germplasm (especially rice) for the development of insect resistant cultivars has been recognized. In this symposium, leading entomologists, plant scientists and policy makers affiliated with the International Plant Genetic Resources Institute (IPGRI), World Bank, International Research Centers, universities, industry, and national programs detail the importance of genetic resources for the development of insect resistant rice, wheat, maize, sorghum, beans, and root and tuber crops. Other invited speakers review the biotechnological properties in conserved germplasm for insect resistant crops and the use of germplasm for basic research in insect-plant interactions.

22-011

GERMPLASM EVALUATION FOR INSECT RESISTANCE IN RICE

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Insects are major biotic constraints limiting the production of rice. Insect resistant cultivars have been bred for use as a major tactic in the development of IPM strategies and they have been shown to be compatible with biological, cultural and chemical control tactics. The world collection of rice germplasm, consisting of more than 100,000 accessions, has been extensively evaluated to identify donor accessions to be used as insect resistant parents in breeding programs at international agricultural research centers in Asia, Latin America and Africa. Commercial high yielding cultivars (30) with multiple resistance to insects and diseases have been developed at the International Rice Research Institute in the Philippines and one variety alone has been grown on 17 million ha in Asia. More than 70% of the commercial rice varieties in Latin America are resistant to the major pest, *Tagosodes orizicolus*. Insect resistant cultivars have contributed to yield stability and the success of the green revolution in rice. Biotechnology holds promise as a means to develop insect resistant rice cultivars where conventional breeding has not been successful. Transgenic rice plants containing the Bt toxin gene are currently in the evaluation stage.

22-012

THE VALUE OF CONSERVED WHEAT GERMPLASM EVALUATED FOR ARTHROPOD RESISTANCE

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Resistance to 10 major arthropod pests of wheat has been identified in evaluations of over 40,000 *Triticum* accessions from several national and international germplasm collections in different countries of the world. Many of the sources of the resistance originated in varieties from southwestern Asia and southeastern Europe.

In the United States alone, the planting of arthropod resistant wheat cultivars currently saves over \$500 million worth of crop production costs and crop losses each year. On a global basis, the value of arthropod resistance in cereal crops is likely to be several billion dollars.

Different wheat varieties have been identified with resistance to the Hessian fly, *Mayetiola destructor* (Say); several aphids, including the greenbug, *Schizaphis graminum*, (Rondani); the Russian wheat aphid *Diuraphis noxia*, (Mordvilko); the grain aphid, *Sitobion avenae* (F.), the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), and the rose grain aphid, *Metopolophium dirhodum* (Walker); the cereal leaf beetle, *Oulema melanopus* (L.), wheat stem sawflies; *Cephus cinctus* Norton and *C. pygmeus* (L.); the sunn pest, *Eurygaster integriceps* Put.; and the wheat curl mite, *Eriophyes tulipae* Keifer.

Resistance breaking biotypes of the Hessian fly and the greenbug have developed in response to numerous different vertical cereal resistance genes. These insect counteradaptations demonstrate the need for continual joint efforts of entomologists and plant breeders to improve cereal varieties for arthropod resistance.

22-014

GENETIC DIVERSITY OF SORGHUM, *SORGHUM BICOLOR* (L) MOENCH, A SOURCE OF INSECT RESISTANT GERMPLASMG. L. Teetes, G. C. Peterson¹, K. F. Nwanzu²

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The early domestication of sorghum in northeastern Africa and subsequent movement to many regions of the world, especially semi-arid, tropical areas, resulted in the evolution of a genetically diverse crop species. This genetic diversity has been exploited in crop improvement programs to increase yield and yield stability by introgressing beneficial genes for many traits including adaptation, grain quality, and biotic and abiotic stress resistance. Major effort has been made to increase the intrinsic ability of sorghum for defense against insect pests through genetic resistance. The photosensitive character of sorghum requires conversion to short, early genotypes for use in temperate regions of the world.

Identifying sorghums resistant to insect pests has been a successful endeavor, and is the focus of research programs by universities, international research centers, and national agricultural research systems. Most of the over 28,000 accessions in the world sorghum collection have been screened for resistance to many sorghum insect pests. Of nearly 50 important insect or mite pest species worldwide that attack sorghum pre- and post-harvest, genetic resistance has been found for about 30% of them. Greatest success in identifying and utilizing resistance has been for greenbug, *Schizaphis graminum* Rondani, sorghum midge, *Stenodiplosis sorghicola* (Coquillett), shoot fly, *Atherigona soccata* (Rondani), and several species of stem borers, armyworms, other aphids, and panicle infesting bugs. Insect resistant sorghums are used as an integrated pest management tactic. Interest in genetically resistant sorghums by industry is increasing as environmental concerns about pesticides increase.

22-013

GERMPLASM EVALUATION FOR INSECT RESISTANCE IN MAIZE

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Insects are major constraints to the production of maize. In much of the world, resistant inbreds or hybrids of maize are grown as the major component of an IPM strategy for maize pests. The world collection of germplasm consists of a large number of entries that have not been totally evaluated for insect resistance, yet it is known that many private companies incorporate resistance into their commercial lines. The private sector companies do not reveal the amount of insect resistance utilized for proprietary reasons. However, public evaluation of widely grown hybrids reveal that seventy to ninety percent of all hybrids grown in the corn belt of the USA have some resistance to one or more insect pests. Multiple insect resistance is being utilized worldwide, especially in many programs affiliated with the international centers in Latin and South America. One or more stem borers are a major problem wherever maize is grown. Biotechnology offers increased tools for the introduction of genetic diversity. Transgenic maize containing the Bt toxin genes are being field tested and commercial hybrids are being marketed in the USA.

22-015

BEAN GERMPLASM RESOURCES FOR INSECT RESISTANCE

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For a crop such as common bean, usually grown by small farmers who can not afford expensive chemicals, host plant resistance may provide an economic, lasting way of overcoming insect and mite attack. Significant progress has been made in the past 15 years in the identification of sources of resistance to leafhoppers, bruchids, pod weevils, Mexican bean beetle, bean stem maggots, pod borers, chrysomelids, and other insects, as well as mites.

This paper reviews advances made in the identification and incorporation of resistance to major pests of beans. It makes emphasis on the value of conserved genetic resources for the development of bean lines and cultivars with resistance or tolerance to insects and mites, provides information on mechanisms and inheritance of resistance and breeding strategies to transfer resistance. It also covers aspects dealing with possible use of molecular markers to facilitate breeding, the value of wild relatives and other species in the improvement of beans, and the future use of host plant resistance as the foundation of integrated pest management systems.

22-016

UTILIZATION OF SWEETPOTATO GENETIC RESOURCES TO DEVELOP INSECT RESISTANCE

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Sweetpotato weevil (*Cylas* spp.; *Euscepes* spp.) is the most destructive insect pest of sweetpotato (*Ipomoea batatas* (L.) Lam.) in the world, occurring from the humid tropics to temperate growing areas. A number of Lepidopteran (*Omphisa anastomosalis*, *Megasiles* spp., *Synanthedon* spp.) and Coleopteran (*Alcidodes* spp. and others) pests can cause severe damage in drier environments. In the US, the WDS complex (wireworm, *Diabrotica*, *Systema*) causes significant damage.

Genetic resources of sweetpotato are extensive; much of the world's germplasm has been collected and is maintained in national and international genebanks. The most significant use of available genetic resources for insect resistance has been for sweetpotato weevil (USA, China, Philippines, Indonesia, India, Taiwan, Nigeria, Peru, Cuba) and for the WDS complex (USA). To date stable, high levels of resistance to sweetpotato weevil have not been found nor have mechanisms of resistance been identified. At present breeding efforts are concentrated on detecting and utilizing low to moderate levels of resistance in a wide array of germplasm. Transgenic sweetpotatoes with genes from unrelated species have been produced and field tested. They may provide an alternative and more effective method for incorporating resistance. Efforts to increase resistance to the WDS complex have been more successful. Moderate levels of resistance were located in populations developed from germplasm accessions and have been incorporated into commercially acceptable material.

22-018

PLANT GENETIC RESOURCES FOR STUDIES IN INSECT-PLANT INTERACTIONS

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Genetic variation in crops has inspired basic investigation of ecological and evolutionary processes ever since Darwin and Mendel. Studies of insect-plant interactions, which have proliferated during the last third of the 20th century, have benefited from the availability of conserved genetic variation in crop plants. Within-species variation in crop plant susceptibility to herbivores has led to a deeper understanding of plant defenses, including glandular and nonglandular trichomes, various secondary compounds and enzymes such as protease inhibitors. Although the primary entomological use of plant genetic resources has been screening for and developing economic resistance to pests, there is potential for more use of these collections for basic research. Study of the genetic basis of defense, including quantitative aspects, genetic correlations of defense and other traits, and the molecular mechanisms of expression is facilitated in genetically conserved crops for which a large number of traits have already been identified and genetically mapped. Conserved germplasm from wild crop relatives provides additional opportunities including study of ecogeographic variation in plant defense against insects. Continuing collection of wild germplasm for crop improvement should be conducted to maximize the value of these for basic research. Thus, ancillary to their primary mission of providing the genetic basis for crop improvement, the germplasm collections of the national and international agricultural centers can continue to contribute to basic research in insect-plant interactions.

22-017

THE POTATO: GENETIC RESOURCES AND INSECT RESISTANCE

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The cultivated potato, *Solanum tuberosum* subsp. *tuberosum* L. is one of approximately 230 potato species (*Solanaceae*, Section *Petota*). This paper discusses the present status of potato breeding for resistance to insects and what the potential is in future. Genes from ten exotic potato species have been incorporated into North American and European potato cultivars, primarily for stress tolerance or disease resistance. Enhanced breeding lines are now being developed specifically for insect resistance. NYL 235-4 incorporates genes from *S. berthaultii* for resistance to Colorado potato beetle, *Leptinotarsa decemlineata* (Say); and other insects. Other examples of germplasm enhancement for insect resistance are the use of *S. pinnatisectum*, *S. commersonii*, *S. tarijense*, *S. sparsipilum*, and *S. sucrense* for potato tuber moth, *Phthorimaea operculella* (Zeller), resistance; and *S. stoloniferum*, *S. spegazzinii*, *S. toralapanum*, *S. chacoense*, *S. polytrichon*, *S. phureja*, and *S. sanctae-rosae* for green peach aphid, *Myzus persicae* (Sulzer), resistance. There are some sources of insect resistance that have been difficult to access using conventional breeding methods, and others that have not yet been accessed. One new method is somatic hybridization, which has brought genes from *S. bulbocastanum* into breeding lines. *Solanum bulbocastanum* has resistance to at least four insects, as well as to root knot nematodes.

22-019

BIOTECHNOLOGICAL APPLICATIONS OF PLANT GENES IN THE PRODUCTION OF INSECT RESISTANT CROPS

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Crops resistant to insect attack offer an alternative strategy of pest control to indiscriminate pesticide usage which has many undesirable effects, including those on the environment and humans. Transgenic plant technology can be a useful tool in producing resistant crops, by introducing novel resistance genes into a plant species.

Several different classes of plant proteins have been shown to be insecticidal towards a range of economically important insect pests from different orders; in some cases a role in the defence of specific plant species against phytophagous insects has been demonstrated. Genes encoding insecticidal proteins have been isolated from various plant species and transferred to crops by plant genetic engineering. Amongst these genes are those that encode protease and α -amylase inhibitors, lectins, and enzymes such as chitinases and lipoxigenases.

Examples of genetically engineered crops expressing insecticidal plant proteins from different plant species, with enhanced resistance to one or more insect pests from the orders Lepidoptera, Homoptera and Coleoptera are presented. The possibility of 'pyramiding' different resistance genes to improve the effectiveness of protection and durability is discussed and exemplified. The viability of this approach to crop protection is considered.

22-020

INSECT RESISTANT CROPS - THE FUTURE

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The conservation and use of genetic resources for the development of sustainable agricultural systems are an internationally recognized priority. Genetic variation for resistance to insect species has been shown in plants, both in natural and managed (agricultural) systems. Thus, genetic resources serve as a source of insect resistant germplasm that are necessary for the development and deployment of resistant cultivars in cropping systems. These resources have been effectively used to develop insect resistant cultivars in rice, wheat, maize, sorghum, beans, and root and tuber crops. Because the emphasis in agricultural systems has been in the selection of one or more genetically resistant traits that are recognized, environmentally stable, compatible with agricultural production systems, and provide an economic level of crop protection, resistance relationships in natural and managed systems must be understood. This will ensure sound approaches to germplasm conservation and subsequent deployment of insect resistant germplasm, and prevent or minimize the development of resistance-breaking insect pest biotypes. The use of biotechnological techniques coupled with an understanding of insect-plant interactions will also provide additional opportunities for conservation genetic resources that can be used to develop insect resistant cultivars in agricultural systems.

22-022

ERADICATION OF THE BOLL WEEVIL IN THE UNITED STATES THROUGH AN AREA-WIDE APPROACH

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The ongoing boll weevil eradication program was initiated in North Carolina, South Carolina, and Virginia in 1977; and in Arizona, California, and contiguous cotton producing areas of Northwest Mexico in 1985. The regional boll weevil eradication programs are cooperative federal-state-cotton producer efforts with a large percentage of the funding coming from the cotton producers within the region. The control measures used in these programs include pheromone trapping to delimit populations, judicious use of chemical treatments and cultural measures such as early stalk destruction and uniform planting. The key elements of these programs are early season treatments of malathion to limit the number of overwintered boll weevils which can colonize cotton, and multiple late season applications of malathion to reduce the number of boll weevils available to enter overwintering. Since program inception, the boll weevil has been eradicated in Arizona, Southern California, and Northwest Mexico, as well as in Virginia, North Carolina, and South Carolina. Eradication is almost completed in Florida, Georgia, and Southern Alabama. In some states (Virginia and North Carolina), the eradication of the boll weevil has resulted in up to a ten-fold increase in cotton acreage, and a 40 to 80 percent reduction in pesticide use. The program currently covers Northern Alabama, Northeast Mississippi, Middle Tennessee, and two areas in Texas. Texas has experienced some secondary pest outbreaks concurrent with the eradication program that are of serious concern to program officials and producers.

22-021

AREA-WIDE PEST MANAGEMENT: CONCEPTUAL, THEORETICAL AND HISTORICAL FRAMEWORK.

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Area-wide pest management is an important strategy for combating the entire population of insect pests within an ecosystem. It is commonly observed that the failure to suppress a mobile pest population in a small fraction of an ecosystem can result in very high economic damage throughout the ecosystem. For example in the case of the desert locust, whose geographical distribution extends from North Africa eastward to the Indian subcontinent, and whose widely separated breeding areas are linked by the seasonal migrations of the pest, the total population must be attacked in an organized manner in order to protect crops. Further the principle has emerged that fairly uniform suppressive pressure applied against the entire population of a pest species throughout the ecosystem results in a considerably greater reduction in its density than is achieved by a higher level of suppression of most but not quite all of the population.

Cases of area-wide pest management that have been of considerable historical importance in entomology include the suppression of the grape phylloxera, *Phylloxera vitifoliae* in Europe through the use of phylloxera-resistant root stocks beginning in 1872, the suppression of the cottony cushion scale, *Icerya purchasi* in California in 1889 by the vedalia beetle, *Rodolia cardinalis*, and suppression of the New World screwworm, *Cochliomyia hominivorax* in the southern USA and northern Mexico from 1962 to about 1975.

In cases where the presence of a pest species is highly damaging to public health or to an industry and where ecological isolation off the production area can be assured, the eradication of the pest population may be desirable economically and ecologically. Examples include the eradication of *Aedes aegypti* from a number of sites early in this century, the eradication of the cattle fever ticks, *Boophilus* spp. from USA during 1906-1943, and the first program to eradicate malaria by suppressing its anopheline vectors that was initiated in Venezuela in 1945.

22-023

AN ASSESSMENT OF THE CODLING MOTH SIT ERADICATION PROGRAM IN BRITISH COLUMBIA, CANADA

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The codling moth, *Cydia pomonella*, is considered the key pest of apples and pears in the fruit growing regions of south-central British Columbia. This region includes about 7,500 Ha of commercial apple and pear production, as well as several urban centers with abundant backyard fruit trees and ornamental crabapple trees. In 1992, after 30 years of research and planning, an eradication program using Sterile Insect Release technology was implemented against this pest. Construction of the mass-rearing facility was completed in 1993 and sterile moths were released into area orchards for the first time in the spring of 1994.

The primary problem facing the Program has been a high wild population, resulting in inadequate overflooding ratios. Contributing to this problem has been poor dispersal activity of mass-reared moths relative to wild moths in cool spring weather. However, a number of operational changes in rearing and handling, coupled with an increased public information campaign and an increased effort from growers brought encouraging results in 1995. Most growers reported low codling moth damage (<0.5%) throughout the season and these reports were verified by field samples at harvest time.

The Program is currently operating only in the southern half of the treatment area. Eradication in some orchards is anticipated by the end of the 1997 growing season, and in all orchards of the south Okanagan by 1999. Clean-up efforts in the north Okanagan are scheduled to begin in 1997, with releases starting in the year 2000. It is hoped that by 2005 the entire area will be codling moth free.

22-024

DEVELOPMENTS IN THE AREAWIDE SUPPRESSION OF CODLING MOTH

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A regional, multi-institutional program to assess, test and implement an integrated strategy for suppression of codling moth populations in fruit orchards was implemented. It was designed to alleviate the impact of neurotoxic pesticides on natural enemies while at the same time continue to control pest populations. This created the opportunity for use of more environmentally friendly control tactics for secondary pests. Five pilot test sites were located, one each in California and Oregon and three in Washington State, to demonstrate the utility of using mating disruption, biocontrol and, in one case, release of sterile moths to control codling moth and secondary pests with a minimum of neurotoxic chemicals. First year results indicated that this strategy could effect control of both codling moth and secondary pests with a greatly reduced amount of pesticides. The pilot programs will continue for four more years. Funding for this program is shared currently by the Agricultural Research Service and the fruit industry. Additional support is provided by State Universities in the three states. A series of research projects impacting the pilot test programs were funded as well. Eventually, this program will be adopted and supported entirely by the growers themselves.

22-026

RECENT DEVELOPMENTS IN CORN ROOTWORM AREA-WIDE MANAGEMENT STRATEGIES IN THE UNITED STATES

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Corn rootworms, primarily the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, and the northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, are among the most economically and environmentally important insects pests of United States maize production systems. Crop losses and control costs attributed to both species approach \$1 billion annually. Soil insecticides targeted at corn rootworm continue to represent the single largest use of insecticide in the United States. Scientists with USDA-ARS and the agricultural experiment stations of several midwestern states developed an alternative management strategy based on suppression of rootworm adults using a semiochemical insecticide-bait. The bait, composed of cucurbitacin, carbaryl, and insecticide carriers, is used to kill beetles before they lay the majority of their eggs and thus prevent economic infestations from occurring if maize is planted in the same field in the successive year. Commercially available baits use 95 to 98% less toxin active ingredient than traditional insecticide management programs.

Semiochemical insecticide-baits have been used successfully to manage corn rootworms at several sites in the United States corn belt. Management of beetles is most effective when done over a relatively large area. Using semiochemical insecticide-baits with other corn rootworm management tactics (crop rotation, biological control, etc.), state-of-the-art population monitoring technology, and new maize management technology will assist implementation of areawide management technology on portions of the estimated 2.5 million ha of maize production with significant corn rootworm populations. Expanded demonstration of this technology in the United States corn belt was initiated during the 1996 growing season and is expected to increase in scope during the next 3-5 years.

22-025

FRUIT FLY (DIPTERA: TEPHRITIDAE) AREA-WIDE INTEGRATED MANAGEMENT PROGRAMMES IN PROGRESS IN THE WORLD

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Tephritid fruit flies are major pests of fruits and vegetables. Because of the high economic value of the crops they attack and the significant damage a few gravid females can cause, the economic threshold for fruit fly damage is very low. In addition, fruit fly capacity for dispersal and survival in marginal and urban areas frustrates individual control efforts at the orchard level. Furthermore, quarantine restrictions impede trade in fresh fruit because of closed export markets or expensive post-harvest facilities needed to disinfest cargoes for shipment.

To overcome the unsustainable and often ineffective fruit fly control resulting from uncoordinated actions by individual producers, growers have been forced to coordinate their control efforts, including in non-commercial areas. Also, because of the quarantine restrictions, whole fruit producing regions have tended towards coordinating fruit fly control for certification of low prevalence or fly-free status.

As a result of the above, fruit fly area-wide management is becoming widely accepted as a necessity by the fruit industry. Area-wide campaigns, coordinated by grower associations or regional/national authorities in a number of countries, integrate various control methods, but often still rely on aerial protein-bait sprays or male-annihilation.

The Sterile Insect Technique (SIT), because of its specificity, environment-friendliness and compatibility with biological control of other fruit pests, is becoming the preferred area-wide management tool for fruit flies. Important successes have been achieved, most recently in Chile and Japan, where all pest fruit flies have been eradicated. Presently, there are area-wide fruit fly SIT control or eradication programmes in progress in Argentina, Australia, Costa Rica, Greece, Guatemala, Mexico, Pakistan, Peru, Philippines, Portugal, Thailand, and the USA, and under consideration or planning in some Mideastern, Maghreb and Central American countries, as well as in South Africa, Sicily, and Uruguay.

22-027

RECENT DEVELOPMENTS IN AN AREA-WIDE, INTEGRATED APPROACH FOR THE MANAGEMENT OF THE COTTON BOLLWORM, *HELICOVERPA ZEA* (LEPIDOPTERA: NOCTUIDAE)

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In the development of an area-wide, integrated approach for the management of the cotton bollworm, *Helicoverpa zea* (Boddie), individual control components are assessed for their potential for preventing or reducing the seasonal increase of bollworm populations. Candidate components include resistant plant varieties, natural enemies, biologicals, and autocidal control. Cultural practices and regulatory mandates also may be important area-wide management strategies.

Recent laboratory and field studies have revealed that many control components are compatible and, when used together, are often synergistic. Also, population models project that program resources divided among several control components may yield greater population suppression than program resources directed at a single control strategy. Current research efforts attempt to identify compatible control strategies, to develop efficient production and application technologies, and to integrate effective control strategies in an area-wide pest management program.

22-028

AREA-WIDE MANAGEMENT OF TOBACCO BUDWORM AND COTTON BOLLWORM WITH AERIALLY -- APPLIED INSECT VIRUSES IN THE UNITED STATES

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Six years of research have shown that aerial application of a baculovirus (NPV) to early season hosts of the tobacco budworm, *Heliothis virescens* (F.), and the cotton bollworm, *Helioverpa zea* (Boddie), resulted in a significant reduction in the numbers of adults that emerge from treated areas and migrate to nearby cotton fields. We estimate that a properly timed treatment with good coverage of host plants with NPV consistently resulted in at least 70% of the insects on these plants dying from the virus as larvae or pupae. Long-range movement into and out of the treated areas is suggested as the cause of our inability to show a significant reduction in the numbers of eggs/larvae on cotton in the treated area compared to the surrounding area. Transfer of this technology to cotton producers in the Mississippi Delta area of the United States may result in future expansions of this program.

22-029

PINK BOLLWORM AREAWIDE MANAGEMENT

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Integrated pest management (IPM) and community-wide participation programs have been important factors in pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) population suppression. Cotton IPM had its beginning with scouting programs that reduced numbers of acre treatments and costs per acre. In the San Joaquin Valley, CA, an areawide program has been conducted since 1968 to prevent establishment of migrating PBW. The program involves pheromone trapping, release of radiation-sterilized moths, cotton plant destruction and plowdown, and mating inhibition. Other large-scale areawide management systems with all available technology for PBW population suppression are in various developmental stages. However, in all PBW-infested cotton areas in Arizona and California, cotton scouting is practiced, and pheromone trapping and boll sampling determine the need for control based on established economic thresholds. Several aspects of PBW biology and ecology contribute to natural population regulation. In early season PBW suicidal emergence, natural enemies and high soil temperatures result in low (0.5 to 1.5 X) population increase. Resistant transgenic cotton (Bollgard™), short season cottons and pheromone-behavioral control are being implemented in varying degrees. In late-season, reducing development of the diapause PBW generation by eliminating host material or destroying diapause larvae using tillage or irrigation techniques are powerful and economical population suppression methods. Stalk shredding, burial of plant debris, disking, plowing and winter irrigation are vital management tools. Incorporating short-season methodology, resistant varieties and good cultural practices on an areawide basis can effectively reduce PBW populations to noneconomic levels.

22-030

THE CASSAVA MEALYBUG *PHENACOCCLUS MANIHOTI* (HOMOPTERA, PSEUDOCOCCIDAE) BIOLOGICAL CONTROL PROGRAM: A MODEL

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The cassava mealybug (CM) *Phenacoccus manihoti* Matile-Ferrero has been introduced accidentally into Africa in the mid seventies. Its rapid spread and devastating damages threatened the major staple of over 200 million people. The CM arrived on planting material that had been introduced illegally, ignoring quarantine regulations banning introduction of vegetative plant material. With the potential to cause famine, the CM introduction caught the attention of governments and the international donor and research communities. In 1979, the International Institute of Tropical Agriculture (IITA) started work in biological control. This led to the creation of the IITA Biological Control Program (BCP) which developed a comprehensive research and training program and collaboration with scientists in Africa, Europe, and the Americas.

The strategy developed for the CM control covered systematic exploration of the likely areas of origin of the pest from southern California to Paraguay; rearing of the most promising natural enemies and detailed taxonomic, biological, and ecological studies in the area of origin and of introduction; their release in Africa over an infested zone one and a half times the area of the United States of America; an analysis of the cropping system and economic impact of the pest and its indigenous and introduced natural enemies and a large scale training and implementation program. The CM biological control program was a very rapid success because of the early discovery of the very efficient parasitoid *Epidinocarsis lopezi* (De Santis) (Hymenoptera, Encyrtidae). Its establishment, rapid spread and impact on the CM, which gave new confidence in biological control to policymakers and donors.

The success of the CM biological control program is due to the unique collaborative arrangements, the introduction of new technologies biological control practitioner had shunned until now. The program has already served as a model for several biological control projects and has left a legacy of trained personnel and infrastructure.

22-031

MANAGEMENT STRATEGIES FOR THE CONTROL OF SILVERLEAF WHITEFLY, *BEMISIA ARGENTIFOLII* (HOMOPTERA: ALEYRODIDAE)N.C. Toscano, N. Prabhaker Castle, T.J. Henneberry¹, S.J. Castle¹Department of Entomology, University of California,
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Control of the silverleaf whitefly in California is dependent upon year-round insecticide use. The overlapping of crops which host whiteflies throughout the annual cropping cycle encourages high whitefly densities. As a result of this and the use of insecticides, there is a potentially high selection pressure for resistance development in the silverleaf whitefly. Because insecticides played an important role in crop protection strategies against this pest, an insecticide resistance management program was instituted.

A resistance monitoring technique using yellow sticky cards sprayed with grower available insecticides provided baseline data for a number of insecticides used for control of *Bemisia argentifolii* in California. Continuous monitoring of the whitefly populations for changes in resistance frequencies in large regions allowed decisions regarding effective management strategies to be formed. Whitefly insecticide resistance monitoring data and management strategies were communicated to farmers and pest control advisors via newsletters, newspapers, and Internet.

22-032

INTEGRATION OF THE STERILE INSECT TECHNIQUE (SIT) INTO SUB-REGIONAL TSETSE AND TRYPA NOSOMOSIS MANAGEMENT CAMPAIGNS

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As the tsetse and trypanosomosis problem is characterized by many interdependencies, any intervention will have a wide range of immediate and longer-term implications. All environmentally acceptable tsetse/trypanosomosis intervention methods have their specific limitations and only a combination of several methods in an integrated, phased approach can effectively advance the establishment of viable agricultural systems. Since the problem affects entire sub-regions, area-wide integrated campaigns should be designed that are largely based on the participation of beneficiaries. This needs comprehensive planning that retains flexibility and a variety of intervention options, including eventual tsetse eradication.

The SIT is the environmentally safest intervention method and can be used even for inaccessible areas (aerial releases). Contrary to other tsetse control methods, the SIT has a unique efficiency pattern: increasing efficiency with decreasing target pest population density. A phased and complementary use of other methods, such as insecticide impregnated targets or the use of pour-on formulations of insecticides on domestic animals, followed by the SIT will have maximum efficiency throughout the intervention campaign. This approach is currently being demonstrated in a campaign to eradicate *Glossina austeni* from Zanzibar. FAO/IAEA has launched an initiative to upgrade the SIT to an economically attractive alternative for integration into area-wide sub-regional intervention campaigns. This involves R&D for the release of at least 500,000 sterile males per week over areas as large as 10,000 to 20,000 km² at a time.

22-034

PRELIMINARY FIELD PREDATION STUDIES OF THE OLIVE MOTH, *PRAYS OLEAE* (BERNARD)(LEPIDOPTERA: YPONOMEUTIDAE) IN GRANADA, SPAIN: A SEROLOGICAL APPROACH.

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The olive moth, *Prays oleae* (Bernard) is one of the principal olive pests. A polyclonal antiserum was raised to aid the study of its associated natural enemy complex. The antiserum was absorbed against the olive bark beetle *Phloeotribus scarabaeoides* (Bernard), to eliminate nonspecific cross reactions. A detection limit was established taking into account background absorbance and the mean 2.5 times the standard deviation of the highest cross reacting species - to guard against false positives. Pheromone trapping and personal observations suggest that the detected predation was probably the olive moth rather than the cross reacting species. This agrees with the observed concordant phenologies of both predator and prey. The results are based on samples from two olive groves in Granada, one abandoned and the other managed, during the months of March through to September in both 1994 and 1995. In total some 20,000 are currently being tested representing over 100 species. Parasitoids and nonpredatory groups were excluded.

22-033

RELATIONSHIPS BETWEEN PHEROMONE TRAP CATCHES OF MALE OLIVE MOTHS, *PRAYS OLEAE* BERN. (LEPIDOPTERA: HYPONOMEUTIDAE), AND FLOWER AND FRUIT INFESTATION BY SUBSEQUENT LARVAL GENERATION

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A three year study (1993-1995) on the relationship between catches of male olive moths, *Prays oleae* Bern., in sexual traps and flower and fruit infestation by subsequent larval generation, was carried out in one olive grove of the Cobrançosa cultivar, at Terra Quente Transmontana (North-eastern Portugal). The degree of *P. oleae* infestation, considered as the percentage of flowers and fruits with hatched eggs, was measured each year in 25 samples collected from each of 25 marked trees, within the experimental plot. Male moths were caught using five Delta pheromone traps, each year.

There was a significant linear relationship between larval infestation and accumulated adult catches. A regression model of the form: $Y = -0.0624 + 0.0002 X$, where X = accumulated adult catches and Y = percent of flower infestation, explained 83.6% of the variance for flower infestation, while a model of the form $Y = 0.0581 + 0.0003 X$ where X = as above and Y = percent of fruit infestation, explained 93.7% of the variance for fruit infestation. These results emphasize the role of sexual traps as a prediction and forecasting method for olive infestation by *P. oleae*.

22-035

OBSERVATIONS ON THE RESISTANCE OF SOME OLIVE CULTIVARS TO *PRAYS OLEAE*.

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During 1995, in an olive grove in southern Sardinia the resistance of 21 cultivars to the carpophagous generation of *Prays oleae* Bern. (olive kernel borer) was studied. The number of eggs laid per olive varied from 0.02 to 0.74, and was higher in table varieties and in trees with lower production. Multiple regression analysis permitted to establish a greater relative contribution of the number of fruits per tree to the estimation of the infestation level. Predators and climatic factors determined an average mortality of 60% of the eggs. The fall of the drupes in the post-setting stage caused an ulterior reduction in the infestation and the percentage of olives with penetrating larvae resulted as 0.4-29%. The autumn drupe fall caused by the kernel borer was 0.1% - 20%, according to the variety. The reduction index, calculated from the ratio between the percentage of infested olives at the end of June and that of the infested fallen olives in autumn, was found to be negatively affected by the average weight of the drupes at harvesting, with values generally greater than 10 for oil varieties. The high resistance found in some cultivars with small drupes (oil producing varieties) is due to both the high fall in the post-setting stage that occurs with greater frequency for infested olives, and to intrinsic factors not yet identified that probably cause larval mortality.

22-036

INFLUENCE OF VARIETY AND MATURATION OF *OLEA EUROPAEA* L. FRUIT, ON *BACTROCERA (DACUS) OLEAE* (GMEL.) (DIPTERA: TEPHRITIDAE) ALCOHOL DEHYDROGENASE ALLELE FREQUENCIES.

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The influence of the variety and the maturation of the host, olive fruit tree, *Olea europaea* L., on the olive fruit fly *Bactrocera (Dacus) oleae* (Gmel.) (Diptera: Tephritidae) was estimated, concerning alcohol dehydrogenase (ADH) allele frequencies.

Wild flies were collected as larvae from infested olives of different varieties from unsprayed olive orchards of Attiki, Greece. The varieties were: Koroneiki (small and large fruit size at an early and late stage of maturity), Megaritikiki, Kalamon and Oleaster. Allele frequencies were based on gel electrophoresis results. In order to evaluate the extent of influence in allele frequencies, chi-squares tests were performed between the different selected populations of olive fruit fly.

The ADH allele frequencies were not affected by the variety of the olive fruit and the stage of its maturity, in spite of the differences observed in the chemical composition of the varieties examined.

22-038

AN INTEGRATED PEST MANAGEMENT APPROACH FOR THE CONTROL OF THE OLIVE PESTS *BACTROCERA OLEAE* AND *PRAYS OLEAE*

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An integrated pest management (IPM) system to control the major olive pests, the olive fruit fly *B. oleae* and the olive moth *P. oleae* was tested in medium size olive orchards. Microbial pesticides, mating disruption and lure and kill methods, were successfully integrated. The results obtained revealed, that one Bt-treatment during the first year of (IPM) application to reduce the larval population in the flower generation of *P. oleae* and continuous application of the mating disruption for three years progressively reduced moth population from one year to the next. Fruit infestation remained at commercially acceptable levels. The lure and kill method is efficient and provides acceptable level of fruit damage in low fruit fly densities, while in high population densities additional control measures are required to keep the infestation within acceptable levels. Despite the fact, that the lure and kill method provides inadequate protection to the olive fruits, in years of high population densities of the fly, it is recommended to the farmers, since the method significantly reduced the number of toxic pesticides applications.

22-037

LOSS ASSESSMENT DUE TO *PRAYS OLEAE* BERN. AND *BACTROCERA OLEAE* GMELIN IN MOURA'S REGION (PORTUGAL). PRELIMINAR RESULTS.

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A study was carried out in 1993, in an olive grove near Moura, to evaluate the losses caused by *Prays oleae* Bern. infestation in the flower and fruit generations and *Bactrocera oleae* Gmelin infestation on the preharvest fruit dropping.

For the *P. oleae* purpose, the number of flowers damaged by larvae was evaluated on marked branches of 25 trees from the Cordovil cultivar. In this year, however, the damage was very low. The fruit dropping from the whole of the tree canopies under observation was recorded weekly from fruit setting to harvesting. The percentage of olives which dropped as a result of *P. oleae* infestation was very high, 62.5% of the dropped fruits. The weight loss due to the olive moth was, on average in the 25 trees, 44.99% of the weight of harvest fruits (mean per tree equal to 10.98 kg).

For the *B. oleae* the damage of the fruits was assessed on 25 trees from the Cordovil cultivar, every two weeks. From each tree a sample of 25 dropped fruits was taken in each assess set.

The yield loss, which was calculated by weighing the fruit drop caused by *B. oleae* from the 25 trees, was on average, 15.3% of the fruit harvested from the canopy.

22-039

OLIVE GROVE ENTOMOFAUNA ANALYSIS AND FRUIT FLY CONTROL STRATEGIES IMPACT.

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Distribution, abundance and seasonal fluctuation of the entomofauna of a coastal olive grove not subject to herbicide treatments and therefore particularly complex from a biocenose point of view were analyzed over a two-year period. In addition, a comparison was made of the quantitative and qualitative effects on the insect community obtained by different insecticide treatments methods ("curative" and "preventive"), strategies ("calendar-based", "targeted") and active ingredient (Dimethoate and Delthamethrin) utilized in "preventive" control against *Bactrocera oleae* (Gmel.).

No substantial differences in qualitative presence of the insects were observed between the two years of sampling. *Diptera Brachicera* (except *B. oleae*), *Rhynchota Homoptera* (mainly *Cicadellidae*) and *Microhymenoptera* were the groups presents constantly, the first two at a very high percentage. At a quantitative level, on the other hand, a marked difference between the total numbers of insects captured was found, in agreement with the infestation level (number of fruit flies) collected. These alternations were a consequence of the different climatic state and olive production level. In spite of these differences the structure of the insect community and the seasonal fluctuation of the groups remained substantially stable.

Treatments appeared to have a quantitative negative impact on the olive-grove entomofauna. This occurred immediately after the period of the treatment but also during the period of rapid population growth. This finding did not differ substantially between the two olive fruit fly control methods ("adulticide" and "larvicide") or between the two different active ingredient or treatment strategies used in the preventive method. However, a more prolonged effect on insect presence was observed in the larvicide trial. Data thereby obtained could be used as biological indicators of the health of the agroecosystem.

22-040

SIDE EFFECT OF THE AIR BAIT TREATMENT AGAINST THE OLIVE FLY AND THE APPLICATION OF ALTERNATIVE METHODS FOR THE OLIVE FLY CONTROL IN CRETE

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The olive fruit fly (*Bactrocera oleae* Gmel.) is the most important insect pest in the olive groves of Greece and specially of Crete. The air bait sprays as well as the improper applications of the ground bait sprays, carried out until today, for the insect control, have caused side effects on the beneficial insects as well as consequences on the whole environment. For the improvement of an integrated control system, the development of the mass trapping system was tested during 1989 - 1993 in different regions of Chania prefecture. The first two years the plywood insecticide treated traps baited with sex attractant and ammonium bicarbonate were tested in olive groves of about 120.000 and 435.000 trees respectively. The comparison between the trap zone and the control (conventional olive groves) was made on the basis of the adult olive flies captures in McPhail traps, on the olive fruit infestation by the olive fly as well as on the beneficial insect population. The olive fly captures were significantly higher in the control compared to the trap zone and the total average number of olive flies was 22,9 and 14,3 respectively. The final olive fly infestation in the olive fruits was little higher in the trap zone than in control. During 1992 the same kind of trap, baited with the same lures but with different arrangement of trap and density of sex pheromone, were tested in olive groves of about 120.000 olive trees. During 1993, 3 different kinds of traps (plywood trap, insecticide paper trap and cloth trap) were tested in olive groves of about 96.000 trees. During these last two years the olive fly captures were significantly higher (almost double) in the control compared with the trap zone and the final olive fly infestation in the olive fruits was the same in the control and the trap zone. The beneficial insect population at the trap zone was higher compared with the air and the ground bait spray zones.

22-042

LURE AND KILL-TARGET DEVICES USED IN SPAIN TO CONTROL OLIVE FLY (B. OLEAE GMEL.).

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In an attempt to have a lure and kill system which was sufficient long in duration, target devices were developed which consisted of olfactory food and sex attractants which lure the insects onto a Killing device composed of cotton cloth treated with an insecticide.

The long-life sex pheromone lure coupled with the ammonium salt lure for females were used as the attractant sources in such targets used at a rate of one per tree. The insecticide used was a pirethroid formulated for good Knock-down and persistence.

Another target device system was tested which consisted of a yellow piece of cloth treated with an insecticide at the start of the field season. One target device was suspended in each olive tree and treated periodically with either food and/or sex pheromone attractants in a sprayable formulation. The food-lure is applied to the cloths when the flies are not sexually active while a microencapsulated sex pheromone and food mixture is applied when the flies are shown to be sexually active from monitoring traps.

22-041

Development of a simulation model for olive fly population management

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A computer simulation model of olive fruit fly (*Bactrocera oleae*) populations was developed for use in IPM programmes. The model is of discrete, deterministic, box-car type, using hourly air temperature as the main driving variable, drupe availability and infestation percentages of first olive fly generation as input variables. The individual development for the summer generation is simulated by an empirical sub-model, function of temperature and fruit maturation rate; for the following generations the sub-model is based on temperature data.

The population abundance is estimated by means of a fecundity sub-model, temperature and age dependent, and by a mortality sub-model based on meteorological data and on published life-tables.

The variability in development and fecundity is generated applying the Montecarlo stochastic method.

The results obtained from simulation were compared with field data on population abundance collected over a ten years period in Sardinia (Italy).

The model is able to accurately predict the phenology, whereas the population abundance is sufficiently simulated for only half of the observed years.

22-043

AUSTRALIAN CITRUS INTEGRATED PEST MANAGEMENT

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Since 1970, effective integrated pest management (IPM) programs have been progressively implemented for the main foliar and/or fruit pests of Australian citrus - scales, mealybugs, and mites, and citrus leafminer (*Phyllocnistis citrella* Stainton). The successes of these programs have led to dramatic reductions in the use of broad-spectrum pesticides. A significant proportion of orchards are not sprayed annually for the control of pests.

Research by State departments of agriculture, universities, and the Commonwealth Scientific and Industrial Research Organisation (CSIRO) laid the foundations for the successes. However, most recent developments have been based on close cooperation between the State departments and private consultants operating as scouts and/or commercial insectaries.

Citrus growers now fund research and development through a levy, and these funds are matched 1:1 by the Australian Government through its Horticultural Research and Development Corporation (HRDC). Voluntary contributions from private companies are also matched by HRDC, and the government's Australian Centre for International Agricultural Research supports cooperative research with developing countries.

Technology transfer is achieved through publications, field days, workshops, and farm visits with government researchers and advisors, and private consultants playing key roles. Technology transfer through regional groups of growers ('Citigroups') is also supported by HRDC, and HRDC funded personnel contribute to industry newsletters. A technology transfer plan is a prerequisite for HRDC funded research. HRDC recently supported publication of a major book 'Australian Citrus IPM' and Australasian Biological Control Inc. also recently published 'The Good Bug Book' with government assistance. Despite the successes, ongoing research and technology transfer is required to address the threat of new pests, the spread of existing pests such as citrus leafminer, and changing practices and climate. Most of the industry is also poorly equipped to properly use IPM compatible chemicals such as petroleum spray oils (PSOs) which play a crucial role in IPM when natural enemies fail or are absent. This critical situation is being addressed.

22-044

BRAZILIAN INTEGRATED INSECT AND MITE PEST MANAGEMENT OVERVIEWS. Gravena¹

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22-046

AN OVERVIEW OF INTEGRATED PEST MANAGEMENT ON CITRUS IN SOUTHERN AFRICA

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Most of the potentially damaging insect pests on citrus in southern Africa have associated complexes of natural enemies capable of effectively regulating pest population levels. These complexes consist of indigenous species and the products of classical biological control projects in combination with the fortuitous introduction of natural enemies through unknown means. There is therefore ample potential for implementation of integrated pest management practices. However, several other factors make profitable and sustainable production of citrus in southern Africa particularly challenging. Heavy reliance on exports to highly sophisticated and distant markets, requires high economic returns to ensure profitability and quality standards are therefore exacting. Furthermore, growers have to deal with a very wide range of highly damaging pests. The existence of particularly problematic pests such as the indigenous thrips *Scirtothrips aurantii* hinders further development of IPM. Likewise, the availability of insect growth regulators for the control of red scale *Aonidiella aurantii*, which has become resistant to organo-phosphates in most of the region, has also detrimentally affected IPM implementation. This has been directly through the impact on biocontrol agents and indirectly through the consequent changes in control strategies adopted for other pests. Requirements of export markets regarding residues and production practices, resistance and the development of augmentative mass releases of natural enemies, are expected to extensively influence IPM in the region in the future.

22-045

INTEGRATED PEST MANAGEMENT IN MEXICAN CITRUS
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México is one of the major citrus-producers countries in the world, with an almost four million ton annual production from 360,000 ha (30% of fruit production). Valencia orange, mexican lime, Marsh grapefruit and Dancy mandarin are the major crops. Four management levels are present in orchards from 11 states.

Citrus rust mite, Texas citrus mite, false spider mite, Mexican fruit fly, citrus leafminer, citrus whitefly and citrus snow scale are the major pests. Besides, 67 insect and mite species are recorded as secondary, occasional or potential pests.

Natural enemies of 30 species are present in different production areas, mainly Aphelinidae, Eulophidae, and Coccinellidae.

A national IPM Program is in progress with fruit flies, in coordination with USDA to control the main four species, including Mexican fruit fly. Although citrus rust mite and other mite species are usually the key pests, no IPM program is working yet.

Citrus leafminer arrived México in 1994. One IPM program is developing now against this important pest. The status of several parasitoids on scales and other Homoptera is under revision, because exploded populations of several pest species are occurring in different states.

22-047

A BIOLOGICALLY BASED IPM PROGRAM FOR CITRUS IN CALIFORNIA'S SAN JOAQUIN VALLEY

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San Joaquin Valley is the major citrus producing region in California. Its proportion of the state's production is increasing as southern California's production is displaced by urbanization. Unlike southern California growers who rely on biologically based IPM, growers in the San Joaquin Valley apply four or five broad-spectrum pesticides annually to suppress their pests. Pesticide resistance in two key pests, reduced pesticide availability, production economics, and public pressure to reduce pesticide use are forcing growers to adopt new pest management practices. These changes involve the development of a biologically-based IPM program. The program is based on augmentative releases of commercially produced *Aphytis melinus* to suppress armored scales, conservation of resident natural enemies to suppress lepidopteran pests and citrus red mite, and a combination of selective pesticides and resident natural enemies to suppress citrus thrips and the other pests. Current research focuses on documenting the efficacy of the *Aphytis* releases and determining the economics of pest control. A complex of natural enemies are responsible for armored scale suppression and the IPM program has cut total pest control costs in half while maintaining or improving fruit quality and production levels.

22-048

INTEGRATED PEST MANAGEMENT FOR CITRUS IN FLORIDA, USA
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In the past 40 to 50 years, the complex 857,000 acre citrus
industry of Florida has evolved into a highly complex mono-
cultural system grown in four major areas of the state. The
regions differ in cultivars grown, in climate and pest
problems. Numerous phytophagous mites, armored and unarmo-
red scales, mealybugs, whiteflies, aphids, weevils, leaf-
miners and some opportunistic insects represent the arthro-
pod fauna common to Florida citrus. Classical biological
control and augmentation of natural enemies within a
defined IPM strategy has been used in some areas of citrus
production today. Basic biology, pest seasonality, sampling
tactics and monitoring has been studied for citrus rust
mite *P. oleivora*, broad mite *P. latus*, citrus red mite, *P.*
citri. Biological control of mites with predators and fungi
has been researched but further research is needed to impro-
ve their persistence and performance in the field.
Success in biological control of armored scales (Florida
red scale, purple scale) has changed their status from
important to minor pests. Generally, citrus snow scale, *U.*
citri, is considered the only armored scale not adequately
controlled by beneficial insects. The unarmored scales,
mealybugs and whiteflies have been the focus of intensive
classical biological control efforts. Lately, integrated
pest management work is focused in citrus root weevils
(*Pachneus* sp., *D. abbreviatus*), citrus leafminer *P.*
citrella and brown citrus aphid (*Toxoptera citricida*).

22-049

THE USE OF *CRYPTOLAEMUS MONTROUZIERI* (MULSANT)
FOR THE CONTROL OF *PLANOCOCCUS CITRI* (RISSO) IN
CRETE-GREECE.

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The citrus mealybug *Planococcus citri* (Risso)
(Coccoidea: Pseudococcidae) is a major pest of
citrus orchards in Crete. In order to reduce
the use of chemical insecticides, experiments
were carried out in Crete, to introduce integra-
ted control methods of the pest. On potted
orange trees, infested with the citrus mealybug
Planococcus citri (Risso), in a greenhouse the
predator *Cryptolaemus montrouzieri* Mulsant was
released in three predator: prey ratios (1:10,
1:15 and 1:30). The effect of *Cryptolaemus mon-*
trouzieri on populations of the citrus mealybug
was also compared with *Nephus reunioni* Furs and
a chemical (methidathion) under controlled
temperature (25-30 C) and relative humidity (55-
70%), where mealybugs were maintained on pumpkins
(*Cucurbita moschata* (Duch)). Five adult beetles of
each predator were released. Methidathion was
applied at a concentration of 0.1%.

The predator *Cryptolaemus montrouzieri* effe-
ctively reduced mealybug populations. The pre-
dator:prey ratio 1:15, in most cases, resulted
in lower mealybug populations. When compared
with *Nephus reunioni*, *Cryptolaemus montrouzieri*
caused a significant reduction in mealybug popu-
lations. In most cases, significant differences
were not detected between *Cryptolaemus montrou-*
zieri and methidathion, which indicates the
effectiveness of the predator *Cryptolaemus*
montrouzieri in the control of the citrus
mealybug populations.

22-050

APPROACHES ON BIOLOGICAL CONTROL OF *DIALEURODES CITRI* (ASHMEAD) IN TURKEY

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Whiteflies are the most serious pests on citrus in Turkey. Up to now
we determined six species: *Dialeurodes citri* (Ashmead), *Parabemisia*
myricae (Kuwana), *Aleurothrixus floccococcus* (Maskell), *Paraleyrodes*
minei Iaccarino, *Bemisia tabaci* Gennadius and *B. afer* Prisner et
Hosney. Two species, *A. floccococcus* and *P. minei* were determined in
Turkey for the first time near the Syrian border in the east
Mediterranean region in August 1994. To date, both species are
restricted to this small area and no further spread was observed. *B.*
tabaci and *B. afer* were only occasionally observed on citrus and
became never a serious problem. *P. myricae* invaded Turkey in 1982,
but was successfully controlled by the introduced parasitoid
Eretmocerus debachi Rose et Rosen within only three years. Since
then, only very few *P. myricae* are found on citrus in Turkey, which
are, however, mostly parasitized.

Presently the most serious pest in Turkey is the citrus whitefly, *D.*
citri, which is attacked by ten indigenous or introduced predators and
two parasitoids, respectively. The predator *Serangium parcesetosum*
Sicard and the parasitoid *Encarsia lahorensis* Howard have been
mass reared and released since 1990 and both species established well
in citrus orchards. *E. lahorensis* was not very effective in controlling
high population densities of *D. citri*. In contrast, *S. parcesetosum*
seems to be a very promising agent in controlling the citrus whitefly.

22-051

MONITORING OF *PLANOCOCCUS CITRI* (RISSO) POPULATIONS WITH PHEROMONE TRAPS

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Monitoring refers to broad, often qualitative, sampling of pests or
their effects used to trigger more precise, quantitative sampling
programs. It includes surveys, trapping and possibly remote
sensing.

In order to test the "Scale trap" (Isagro-EniChem, Italy) system,
with dispensers baited with 0.25 mg of the synthetic sex
pheromone of the citrus mealybug (CM), *Planococcus citri*
(Risso), in the monitoring of its populations, a study was
conducted in the South of Portugal, during 1991-94. Trapped
males were counted weekly.

The period of male activity began between 10 and 31 of March and
ended between 26 of December and 13 of January. Only the first
two male flights were well defined, but just the second was
detected in all the four plots studied. The first male flight reached
its maximal between 23 of March and 27 of May and the second
between 9 of June and 12 of July. The data suggest that the
phenology of the CM in Faro and Tavira (Algarve) evolve 7 to 15
days earlier than in Setúbal.

The possibility of using "Scale trap" as a tool to initiate sampling
of fruits and to define the opportunity of intervention either in
biological control (e.g., releases of *Leptomastix dactylopii* How.)
or in chemical control of CM is discussed.

22-052

THE DAMAGE CAUSED BY *PLANOCOCCUS CITRI* (RISSE) ON CITRUS GROVES

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The citrus mealybug, *Planococcus citri* (Risso), is usually considered a key pest of citrus groves in several countries, including Portugal, due the damage caused by this species.

There is a lack of understanding in which way the citrus production components are affected by the activity of the citrus mealybug, eventhought the already existing knowlegde about the different types of damage caused by this pest.

The results of several studies carried out in Portugal concerning the damage caused by the citrus mealybug on sweet orange groves, showed that only medium to high population densities caused a significant reduction of production, both in fruit weight and fruit size. However, the settlement of low densities of the citrus mealybug colonies affected fruit external appearance, caused commercial depreciation, due to fruit coloration, fruit splitting and presence of clorotic spots. On the other hand, in terms of commercial depreciation it seems that the number of fruits infested with citrus mealybug colonies is more important than the dimension of the colonie settled on the fruit. More than the importance of damage caused by the citrus mealybug on citrus groves, this fact can also affect the risk estimate and economic threshold for *P. citri*.

22-054

EVOLUTION OF THE POPULATION OF THE CITRUS LEAF MINER AND ITS PARASITIDS DURING 1995 IN VALENCIA (SPAIN)

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The level of new flushes and the population density of the Citrus Leaf Miner (CLM) *Phyllocnistis citrella* Stainton and its parasitoids has been studied in a Navel orange orchard from Catadau, Valencia (Spain) during 1995. Out of 6 periods of new flushes, only 2 bear a high number of shoots per tree, in Spring (from February to April) and at the end of the Summer (August-September). CLM populations are very low or absent in Spring and reach high levels (3-4 mines per leaf) from June to October, so the main damage to the new vegetation of the trees is produced in August and September. The CLM developing stage was closely related with the lenght of the leaf, and not with date or length of the shoot. The percent parasitism by *Pnigalio* sp. increases steadily, being below 10% between May and July, from 10 to 20% in August and September, and above 30% in October. Host-feeding was also observed in this parasitoid. The number of adults of CLM captured in sticky traps of several colors was low and without differences. *Sympiesis gordius* (Walker) and *Sympiesis gregori* Boucek, parasitoids of apple leaf miners, were released in the orchard.

22-053

AUTOCTONOUS PARASITIDS OF THE CITRUS LEAF MINER IN VALENCIA (SPAIN)

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Thirty groves were sampled between September and November 1995 in the Citrus area around Valencia (Spain), by collecting and observing at the laboratory 100 Citrus Leaf Miner (CLM) late stages, and placing shoots in evolutionaries for parasitoid adults. All the orchards had parasitoids, and the rate of active parasitism was very variable, ranging from 10% to 60%, without much relationship with previous history of chemical sprays in the grove. Parasitism increases with time as Autumn progresses. The five species of parasitoids identified were, from more to less abundant, *Pnigalio* sp., *Cirrospilus pictus* Nees, *Cirrospilus vittatus* Walker, *Sympiesis gregori* Boucek and *Chrysocharis* sp. *Pnigalio* sp. is the overall predominant species in the groves in Summer, being gradually replaced in Autumn by both species of *Cirrospilus*. It parasitizes mostly L4, but also L3 and pupae, and its ratio male:female is near 2:1. Both species of *Cirrospilus* are parasitoids of L3 and their male:female ratio is 1:1. They can act incidentally as hiperparasitoids of *Pnigalio* sp.

22-055

ANALYSIS OF A REPRESENTATIVE EXPERIMENT OF BIOLOGICAL AND IPM ON CITRUS ORCHARDS IN EASTERN SICILY

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The results of a research carried out within a big project of IPM applied all through the 1995 in the Sicilian Citrus cultivation with the U.E. funds (C.E.E. Reg. 2078/92, A1).

In this context, 172 variously cultivated firms were investigated (i.e. orange, clementine, tangerine) and placed in the Siracusa, Catania and Messina provinces. The total examined surface is 498.48 ha wide, with a firm dimension varying between 0.13 and 21.90 ha. The biggest entomological problems are represented by aphids (*Aphis gossypii* Glover, *Toxoptera aurantii* B.d.F. and *Aphis spiraeicola* Patch), Citrus Leaf Miner (*Phyllocnistis citrella* Stainton), Mediterranean Fruit Fly (*Ceratitis capitata* Wied.) and Citrus Mealybug (*Planococcus citri* Risso) infestations; the pathological problems are represented by *Phytophthora* sp.

With regard to the new strategy the 12.20 % of the firms has employed beneficials organisms (*Leptomastix dactylopii* Howard and *Cryptolaemus montrouzieri* (Mulsant)) and mineral oils, generally sprayed in the wintertime, the 10.46 % only mineral oils, the 27.90% has utilized only pesticides (but not minerals oils), the 34.29 % has employed during the year mineral oils and pesticides and the 15.11 % of the firms has not treated. The authors discussed these results on the technical, economical, ecological and toxicological outlines.

22-056

CONTROL MEASURES OF *LOPHOLEUCASPIS JAPONICA* COCKERELL (HOMOPTERA:COCCINEA) THROUGH INTEGRATED CITRUS PESTS MANAGEMENTE.S.Tabatadze, V.A.Yasnosh¹

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Japanese scale, *Lopholeucaspis japonica* Ckll. is one of the main pest on citrus plants in the Black Sea coast of Georgia. At present the indigenous natural enemies are great value in controlling this pest. Natural enemies complex includes mainly the aphelinid parasitoids, predatory coccinellids, mites, spiders and also new pathogenic fungus *Aschersonia* sp., unknown earlier in the Caucasus.

The economical threshold of scale is 20 - 25 scales/cm² of trunk or brunches. The criteria of the natural enemies efficiency is 50% of scale population destroying by parasitoides or predators. These indices were used in order to provide the rational control measures. If it is necessary the sprays by mineral oil (1% in summer and 2% in other periods) or by 0,5 % - 1% applaud against the first and second instar larvae are recommended.

22-058

SUCCESSFUL CONTROL OF THE MEDITERRANEAN FRUIT FLY *CERATITIS CAPITATA* (WIED.) (DIPTERA: TEPHRITIDAE) BY MASS TRAPPING METHOD, IN AN ORANGE ORCHARD IN GREECE.

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The application of the mass trapping method for two consecutive years, protected successfully from medflies an orange orchard of 600 trees. Two types of Lure and Kill traps were applied. A trap of the reversed cup type baited with Trimedlure which killed male flies and a modified plastic McPhail trap baited with food attractant (hydrolysed protein) which killed female flies. Medflies attracted in both traps were killed by an insecticide layer put inside the traps. Traps installed in both years at the beginning of September at a density of one trap every second tree for the males and one trap every third tree for the females, decreased in the monitoring traps the natural population of males from 34 to 0.5 and 38 to 0.5 flies/trap and females from 38 to 2 and 72 to 0.9 flies/trap the first and the second year respectively, within 20 days after installation. The low catches in the monitoring traps remained till the end of December. The attractants in the two types of traps were only once replaced during the period of application (after 40 days), while the killing factor was not renewed.

Samples of fruit collected from the orchard, exhibited very low infestation (one pupa was found in 150 fruits and a few oviposition stings) for the first year, while the second year no pupae or stings were found in the samples collected.

Since, no sprays with insecticides were applied in the orchard for two consecutive years, the release of the parasitoid *Cales noacki* (Hymenoptera) for control of the white fly *Aleurothrixus floccosus* Maskell (Homoptera) was made possible.

22-057

MANAGEMENT OF CITRUS PESTS IN EGYPT : FROM RESEARCH TO PRACTICE

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A successful natural control of the citrus pests was achieved after 3 years of discontinuing pesticide applications in an orange orchard. The population of the citrus brown mite initially increased to damaging levels after discontinuing pesticides, then declined and was maintained at a very low level. This coincided with a gradual increase in the populations of the predacious mites. Regarding other citrus pests, the populations of the fruit fly and aphids were maintained also at low levels, but scale insects increased to a noticeable number at the third year. Thereafter, a pest management program based on maximising the role of natural enemies and minimal use of pesticides was applied to maintain the citrus key pests at low levels, but the question now is how can we mitigate damage caused by the citrus leafminer invaded Egypt citrus in 1994.

22-059

MEDITERRANEAN FRUIT FLY INTEGRATED CONTROL: COMBINATION OF GENETIC SEXING STERILIZED MALES WITH LURE AND KILL OF WILD FLIES

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In a pilot field application on 5,000 citrus in the island of Crete, successive weekly releases of about one million gamma-sterilized medfly males were applied from June till December. The test area was protected against wild medfly immigration with lure and kill trap "barriers" of parapheromone and food attractant. Before releases, male parapheromone lure and kill was applied to minimize wild male numbers in the test area.

The above method produced substantial protection of fruits from medfly, as compared to controls and previous year. No insecticides were applied in the test area against the medfly.

22-060

COMPARISON OF *ANAGYRUS SAWADADII* ISHII AND *LEPTOMASTIX DACTYLOPII* HOWARD (HYMENOPTERA : ENCYRTIDAE) PARASITIZING IN THE CITRUS MEALYBUG, *PLANOCOCCUS CITRI* (RISSO) (HOMOPTERA : PSEUDOCOCCIDAE)

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Laboratory experiments were conducted to study the relationship between the citrus mealybug, *Planococcus citri* (Risso) and its two endoparasitoids, *Anagyrus sawadadii* Ishii and *Leptomastix dactylopii* Howard. In choice tests, *A. sawadadii* parasitized on the first and the second instar nymphs of *P. citri* and produced 4.6 and 14.3 progeny, with sex-ratio of 28.6% and 41.9%, respectively. *L. dactylopii* parasitized on the third instar nymphs and the adult of *P. citri* and emerged 4.3 and 8.5 progeny, with sex-ratio of 34.6% and 39.2%, respectively. In no-choice tests, *A. sawadadii* parasitized on the first instar nymphs and the second instar nymphs of *P. citri* and had 12.9 and 22.7, with sex-ratio of 16.9% and 33.1%, respectively. *L. dactylopii* parasitized on the third instar nymphs and the adult of *P. citri* and emerged 7.5 and 19.1 progeny, with sex-ratio of 28.9% and 38.5%, respectively. Developmental duration of *A. sawadadii* in the first instar nymphs of *P. citri* was 30.4 days. *L. dactylopii* parasitized the third instar nymphs of *P. citri* took 21.8 days to develop that was longer than that of the adult of 19.7 days. Adult longevity at 20°C, 25°C, 30°C and 35°C was 35.8, 20.6, 13.8 and 7.7 days for *A. sawadadii*, and 40.9, 24.9, 15.7 and 7.3 days for *L. dactylopii*, respectively. The impact of regulation was demonstrated through parasitoid exclusion experiment: *P. citri* cohorts exposed to these two species of parasitoids for 6 months, number of mealybug on pumpkin were 0.12/cm², whereas cohorts protected from parasitoid, number of mealybug on pumpkin were 0.78/cm².

22-062

DEVELOPMENT OF EGGPLANT VARIETAL RESISTANCE TO INSECTS AND DISEASES VIA PLANT BREEDING

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Eggplant (*Solanum melongena* L.) is an important Solanaceous crop mainly cultivated in the tropical, Asian and European regions. The major objectives of breeding are the development of high-quality, pests and diseases resistant varieties. In the countries where intensive and successive cropping is practised the main important goal is to develop varieties resistant to soil-born diseases (fungi, bacteria and nematodes), insects, mites and fruit rot.

Incorporation of resistance genes into eggplant cultivars has been, so far, very limited because of lacking of desirable traits into the eggplant gene pool or sexual incompatibility with resistant wild related species.

The present communication will review the source of resistance genes to pest and diseases available both in the eggplant gene pool and in the wild *Solanum* relatives. Taking into account the genetic determinism of the resistance traits, the possible strategies for eggplant resistance breeding will be discussed with emphasis on approaches based on integration of classical breeding methods (crosses and selection) with biotechnological ones (anther culture, somaclonal variation, genetic transformation and protoplast fusion).

Experimental evidence on breeding for resistance against some soil-born diseases and pests using such approaches will be presented.

22-061

THE CITRUS LEAF-MINER, *PHYLLOCNISTIS CITRELLA* STANTON, IN SICILY: DEVELOPMENT, DAMAGES AND STRATEGIES OF CONTROL

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The authors relate on the sudden widespread of the infestation of *Phyllocnistis citrella* Stanton all over Sicily during summer 1995 and on its development during Autumn and Winter 1995-96.

The study goes on to review briefly the damages on the infestation and its economics relevance.

The authors then go on to discuss the negative results of attempts to control the infestation by use of different pesticides and to discuss the wider problem of integrated control of the infestation and the mechanical, physical techniques related to it. They believe the conclusions that in the case of yielding citrus crops the best solution can be found in the employment of biological control by the indigenous entomophagy and by the introduction of some exogenous entomophagus. In the Eastern coast of Sicily we have found *Pnigalio mediterraneus* Ferriere, *Cirrospilus pictus* Nees and *C. vittatus* Walker, where in the western one Liotta *et al.* (1966) have noticed the presence of nine autoctonus species: *Cirrospilus diallus* Walker, *C. pictus*, *Ratzeburgiola incompleta* Boucek, *Pnigalio* spp. (three species), *Apotetrastichus sericothorax* (Szelényi), *Apotetrastichus* sp., *Teleopterus exsias* (Walker), *Chrysocharis* sp., *Neochrysocharis* sp. The last three species were unnoted as parasitoids of *Ph. citrella*.

In the case of plants of the nurseries and also for ornamental citrus little plants the Authors suggest the use of mechanical control via the isolation in structures covered by screening nets.

Topgrafted plantings and young plantings should be protected by use of chemical control; but better by the use of sucks of screening net, in which each plant must be imprisoned. Biological control by predators can be applied inside them.

22-063

MUTAGENESIS OF *BACILLUS THURINGIENSIS* GENES TO ENHANCE EXPRESSION IN EGGPLANT

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Bacillus thuringiensis (Bt) genes have been widely used to confer insect resistance in transgenic plants. The expression level of wild type (wt) Bt genes in plants was indeed very low due to the presence, in the nucleotide sequence, of potential regulatory signals for eukaryotes and to an unfavorable codon usage in plants. In this work a modified version of the Bt gene Bt43, belonging to the CryIII class (Coleoptera-specific genes) and active against Colorado Potato Beetle (CPB), was used to confer resistance to this target pest in transgenic eggplant.

The Bt43 gene was modified using a Polymerase Chain Reaction approach. Most of the destabilizing sequences were replaced and the codon usage was adjusted to more closely resemble that found in plant genes.

The homology between the Bt43 the modified gene (BtM) was of 84%. The BtM gene was transferred in eggplant via *Agrobacterium tumefaciens*. The introduction of neutral translationally mutations in the Bt43 coding region resulted in a significant increase of its expression level at mRNA and protein level. Transgenic eggplants showing high resistance against CPB were obtained.

22-064

ENGINEERING TRANSGENIC EGGPLANT (*SOLANUM MELONGENA* L.) RESISTANT TO THE COLORADO POTATO BEETLE (CPB, *LEPTINOTARSA DECEMLINEATA* SAY)

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A chimeric construct, containing the synthetic *cryIIIA* (*Btt*) gene, the NPTII selectable marker and the *uidA* reporter gene, was incorporated via *Agrobacterium tumefaciens* into eggplant, variety Hibush. The synthetic *cryIIIA* gene, altered at the nucleotide level without changing the amino acids of the toxic protein by Dr. J. Kemp of New Mexico State University, Las Cruces, is adapted for high expression in plant cells. To verify the transgenic status, GUS assays were performed on over 300 plants, from which 185 were confirmed to be transgenic. Physical incorporation of the chimeric construct was further confirmed by Southern analysis of about 30 transgenic plants; both single and multiple site incorporation of the *Btt* gene were found. Resistance to CPB was assessed by: a) placing egg masses of CPB on leaves of plants grown in the growth chamber; b) placing first-instar larvae on detached leaves; c) observing 173 transgenic plants under field conditions. Approximately 60% of the transgenic plants displayed a very high level of resistance to CPB. No larvae survived on the resistant plants longer than 50-60 hours after hatching. Upon selfing, the transgenic plants with a single construct segregate in the S₁ generation in a mendelian fashion.

22-066

PREDICTING DURABILITY OF COLORADO POTATO BEETLE RESISTANT EGGPLANT GERMPLASM

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A simulation model was developed to predict the possibility of adaptation by *Leptinotarsa decemlineata* Say to CryIIIB toxin expressed in transgenic eggplant. Two different field deployment strategies were analyzed. In case of completely transgenic fields, a fast buildup of resistance to the toxin by the Colorado Potato Beetle is expected (frequency of resistance allele will overcome 0.5 in the second year of cultivation), even if the plants express moderate levels of toxins. A mixed planting of transgenic and non transgenic clones could, instead, allow to preserve a longlasting efficacy of the germplasm. The main features of the beetle's biology leading to these results in mixed fields are:

- a) fitness costs are associated to the adaptation of *Leptinotarsa decemlineata* to the CryIIIB toxin;
- b) migration of adult beetles from harvesting potato fields into eggplant fields constantly happens late in the growing season. This causes a sensitive reduction of the allele frequency in the beetle population.

22-065

RESISTANT MANAGEMENT PRACTICES FOR COLORADO POTATO BEETLE (COLEOPTERA: CHRYSOMELIDAE) IN VEGETABLE CROPS

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Transgenic potatoes containing δ -endotoxin expressing genes of *Bacillus thuringiensis*, and the systemic/contact insecticide, imidacloprid (Admire 240FS, Bayer Corp.), are highly effective against Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say). Because of the season long constitutive expression of the protein toxin and the systemic action of imidacloprid, these "high dose" strategies exert strong selection pressure which may result in rapid pest adaptation. Several resistant management practices were developed to maximize the long-term utility of these novel products. Bioassay techniques and baseline levels of CPB susceptibility to the purified δ -endotoxin and imidacloprid were established as a reference for tracking resistance development. Seed mixtures, in which nontransgenic plants were randomly interspersed with transgenic plants within a field, were evaluated as a refuge strategy to maximize the probability that resistant CPB will mate with susceptible individuals and produce susceptible offspring. Two practical approaches for creating refuges with soil applications of imidacloprid were also tested: perimeter barriers and row mixtures. These spatial applications of treated and untreated plants in a field significantly reduced costs over whole-field treatments and provided a refuge so that some portion of the CPB population was not subjected to selection.

22-067

INTERPLANTING WITH SELECTED FLOWERS TO ENHANCE PARASITOID & PREDATOR ABUNDANCE.

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Interplanting with flowering herbaceous plants increases parasitoid and predator efficacy in agroecosystems. However, few details are available on the compatibility of these insect's foraging ability with floral architecture (=the spatial relationship of the nectary with other floral parts). Here we show that laboratory evaluations of parasitoid and predator foraging performance on flowers with disparate floral architectures can be used to select the flowers that will be most successful in promoting beneficial insect abundance and species diversity in agroecosystems. We focused on the egg parasitoid *Edovum puttleri* (Hymenoptera: Eulophidae) (EP) and major predators of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), in commercial eggplant fields in New Jersey (USA).

Laboratory evaluations of flowers with disparate floral architectures demonstrated that EP can forage in flowers with exposed nectaries ("suitable" floral hosts) but cannot forage in flowers with partially-hidden nectaries ("unsuitable" floral hosts); while predators such as *Coleomegilla maculata* (Coleoptera:Coccinellidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae) can forage on flowers with both exposed and partially--hidden nectaries.

Preliminary studies in eggplant fields interplanted with selected flowers indicated that the presence of "suitable" flowers significantly increased predator abundance with a corresponding increase in CPB egg mass and larvae mortality.

22-068

BIOCONTROL OF ARTHROPOD PESTS IN EGGPLANT IN ITALY

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The arthropod pests of eggplant in Italy are approximately 10 species. In northern Italy, the Colorado potato beetle (CPB) *Leptinotarsa decemlineata* (Say) (Coleoptera Chrysomelidae) is the key pest. Before the registration of preparations based on *Bacillus thuringiensis* subsp. *tenebrionis* (B.t.t.), trials were carried out on biocontrol using seasonal inoculative releases of *Edovum puttleri* Grissell (Hymenoptera Eulophidae) (EP). For several years we reared this egg parasitoid on CPB. Releases of EP pupae gave satisfactory control of CPB infestation in eggplant grown under plastic tunnels. Mass production of EP was not developed due to high labour cost for the CPB and EP rearings. Another reason was also the success of CPB control by means of B.t.t. Five cases involving others arthropod pests and biocontrol agents are listed. (1) Spidermite, *Tetranychus urticae* Koch (Acarina Tetranychidae) is usually controlled by only one release of *Phytoseiulus persimilis* Ath.Hnr. (Acarina Phytoseiidae). (2) Whiteflies are more dangerous in southern Italy; their control can be achieved by releasing the predator *Macrolophus caliginosus* Wagn. (Rhynchota Miridae) that attacks both *Trialeurodes vaporariorum* (Westw.) and *Bemisia* spp. (Rhynchota Aleyrodidae). Releases of the two parasitoids *Encarsia formosa* Gah. and *Eretmocerus mundus* Merc. (Hymenoptera Aphelinidae) can improve the efficiency of the predator used alone. (3) *Frankliniella occidentalis* (Perg.) (Thysanoptera Thripidae) and other thrips species control is conducted releasing *Orius laevigatus* (Fieb.) (Rhynchota Anthocoridae) as soon as the first thrips captures in blue sticky traps are made. (4) *Aphis gossypii* Glover, *Myzus persicae* (Sultz.), *Macrosiphum euphorbiae* (Thom.) (Rhynchota Aphididae), in relation to location and rate of infestation can be controlled, respectively, by releases of *Aphidius colemani* Viereck and/or *Lysiphlebus testaceipes* Cres. (Hymenoptera Aphididae), *Chrysoperla carnea* (Steph.) (Neuroptera Chrysopidae), and/or *Harmonia axyridis* Pallas (Coleoptera Coccinellidae). (5) *Liriomyza* spp. (Diptera Agromyzidae) are well controlled by releases of *Diglyphus isaea* (Walker) (Hymenoptera Eulophidae). In some areas, the implementation of biocontrol in eggplant will be particularly difficult due to the necessity of releasing many species of natural enemies. However, farmers can choose this strategy since all the biocontrol agents herein listed are sold in Italy by biofactories.

22-069

DEVELOPMENT AND USAGE OF SEQUENTIAL SAMPLING IN A BIOLOGICAL CONTROL INTENSIVE PEST MANAGEMENT PROGRAM

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Sequential sampling has been used in agricultural systems to rapidly estimate pest densities relative to the need for instituting control measures for many years. In the mid to late 1980's a biological control intensive pest management (BCIPM) program targeting the Colorado potato beetle (CPB) was implemented in New Jersey for eggplant. This program included releases of the egg parasitoid, *Edovum puttleri* for the suppression of beetle populations; monitoring of fields; and recommendations for chemical sprays. Initially, CPB populations were monitored twice a week by determining the mean number of eggmasses, small larvae (1st and 2nd instars), large larvae (3rd and 4th instars) and adults found on 100 plants selected from within each field. Decisions were then made regarding parasitoid effectiveness and the need for insecticides. This type of time intensive monitoring program, however, limited the number of hectares that could be managed by the program. Due to this, two sequential sampling programs, one for eggmasses and a second for adult and larvae, were developed. The sampling program for adults and larvae utilizes a single economic threshold that accounts for differences in the consumption rates between life stages. The placement of larvae and adults on an equivalent basis accelerates monitoring by allowing the sampler to count individuals independent of life stage. Both sampling programs were field tested prior to release to the BCIPM program and have been used since 1992.

22-070

DEVELOPMENT AND IMPLEMENTATION OF A BIOLOGICAL CONTROL INTENSIVE PEST MANAGEMENT PROGRAM IN EGGPLANT

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The methods and techniques of implementing a biological control intensive pest management program in eggplant are discussed. The biology and behavior of *Edovum puttleri* a parasitoid of the Colorado potato beetle are presented as a major tool in preventing plant damage.

22-071

ERADICATION OF *CYDIA POMONELLA* (L.) FROM WESTERN AUSTRALIA

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Cydia pomonella (L.) is a worldwide pest of apples and pears. Japan and Western Australia are the only two major apple growing regions of the world free from the pest. In January 1993, *C. pomonella* was found at Bridgetown in the south-west of Western Australia and three months later further south at Albany. Following the first discovery, an eradication campaign was commenced based on a protocol developed through a consensus process during the early weeks of the campaign. The protocol, which needed only slight modification for the Albany eradication, had two aims: (1) local containment of the outbreaks and (2) surveillance of other fruit growing districts and urban areas to ensure the region was returned to its former codling moth free status, an important trade advantage.

The eradication campaign had four components: regulation, sanitation, pest monitoring and public education. Regulatory powers allowed the affected areas to be quarantined, restricted the movement of any fruit likely to be a host of the insect and allowed likely sources of infestation to be checked. Fruit, trees, and in some cases whole orchards, were removed and any material likely to harbour the pest burned and buried. A cover spray program was required on commercial orchards. Sex pheromone traps were used to monitor adults in the infested areas and a grid of traps was established elsewhere in the state to ensure the pest had not moved from the quarantined areas. The presence of larvae was monitored by examining trees, dissecting fruit and by trapping with trunk bands. An extensive public awareness campaign backed up the field operations.

By December 1995, *C. pomonella* had not been detected anywhere in Western Australia for two years and it was concluded that eradication was successful.

22-072

INTEGRATED PEST MANAGEMENT IN AUSTRALIAN APPLE ORCHARDS

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The Australian apple industry has advanced towards integrated pest management (IPM) in response to consumer concern about use of pesticides on fruit crops and to the development of resistance in several pests. The need for an IPM program was foreseen in the mid 1960s when a national research project was undertaken which provided valuable information on the ecology of the apple pest complex throughout southern Australia. This was followed by integrated control of pest mites based on phytoseiid mites with the strategic use of acaricides and the avoidance of pesticides toxic to predators. Industry funding for research was formalised in 1988 and since then the Australian apple and pear industry has made a substantial financial commitment to IPM related research. Private industry, including agricultural chemical companies and producers of biological control agents, has also contributed to the IPM research and development effort.

The current focus is on the control of *Cydia pomonella* (L.), codling moth, the key pest in apple orchards in south-east mainland Australia, and the native leafroller *Epiphyas postvittana* (Walker) which is a problem in the cooler south. Conventional control relies on a program of the insecticide azinphos-methyl over 4-5 months of the growing season. One of the insect growth regulator compounds, fenoxycarb, was introduced as an IPM approach to lepidoptera control in 1991. One advantage of this approach has been reduction (after three seasons without azinphos-methyl) in the pest status of *Eriosoma lanigerum* (Hausmann). Other "soft" insecticide alternatives to azinphos-methyl are under trial. Recent research has evaluated mating disruption for both *C. pomonella* and *E. postvittana*. The value of supplementary sprays of azinphos-methyl or fenoxycarb with mating disruption of *C. pomonella* has been examined. Codling moth granulosus virus and an insect parasitic nematode have been investigated as biological control alternatives for *C. pomonella* compatible with IPM.

22-074

THE PEST ARTHROPOD MANAGEMENT IN ORCHARDS AT HILLY RESERVOIR AND OTHER DISTRICT NEAR WATER RESOURCES

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During 1992-1993, the main pest arthropods and management for them in orchard where were near reservoir, river and sea, at Laiyang, Gaomi and Penglai, Shandong, China had been studied. It was defined that: 1 The arthropod community structure at different times in apple orchard. The leading pest species were *Aphis spiraeocla*, *Tetranychus viennensis*, *Panonychus ulmi*, *Carpocapsa nipponensis*, *Spilonota lechriaspis*, *Myzus malisuctus* and *Heliothis armigera*. 2 The spatial distribution of *Spilonota lechriaspis* larvae and the influence of releasing *Trichogramma*. The spatial distribution of overwintering and first generation *S. lechriaspis* larvae, pupas and so on were negative binomial distribution. 3 The control effect of Clofentezine to *Tetranychus viennensis* and *Panonychus ulmi* were more than 90% during 20-30d post controlling. 4 The effect of applying *Trichogramma dendrolimi* to apple and pear orchards were 46.7-78.8% to the eggs of *Adoxophyes orana*. 5 The effect of controlling *Carpocapsa nipponensis* by *Steinernema carpocapsae* were 40-80% when 0.075-0.15 billion nematodes/ha. 6 The IPM technology of the pest arthropod in apple orchard that took released and protected natural enemy as the principal thing was given.

22-073

ACHIEVING IMPACT IN STONE FRUIT INTEGRATED PEST MANAGEMENT PROGRAMS IN NEW JERSEY, USA

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A statewide program combined scouting for all arthropods and diseases, nematode detection, and monitoring plant nutrition through soil and leaf tissue analyses. On farm research plots demonstrated mating disruption practices for *Grapholita molesta* (Busck) (OFM) and *Synanthedon exitiosa* (Say) (PTB) and degree day based modeling for *G. molesta* and *Platynota idaeusalis* (Walker) (TABM). Technology was transferred through scouting reports, newsletters, grower meetings, telephone recordings, fax, phone and consultations. Commercial growers producing 65% of the NJ peach and nectarine crop (\$19 million in '95) have enrolled in the program during the last 4 years.

Grower practices included the use of field scouting, threshold levels, alternate middle spraying, biological control of mites, selective pesticides at reduced rates, degree day models, mating disruption, and adherence to fertilizer and nematicide recommendations.

Pesticide inputs varied, but showed overall decreases, especially insecticides. Intensive IPM practitioners saved \$250/ha compared to standard state recommendations. Total farm use of formulated pesticides ranged from 110 kg/ha to 188 kg/ha in 1995. Insecticide use ranged from 11 to 24 kg/ha. Degree day modeling combined with scouting permitted a 40% reduction in insecticide use. Use of mating disruption permitted a 76% reduction in insecticide use from 12.8 kg/ha to 3.1 kg/ha where insecticide was used only for first generation OFM and second generation TABM. Fruit quality averaged over 83% clean fruit.

22-075

APPLE PRODUCTION WITHOUT THE INPUT OF NEUROACTIVE INSECTICIDES

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This was the initial year of a five year comparison of the ecology and economics of Delicious apple orchards managed without using neuroactive insecticides (NNAI) or managed conventionally (CONV). Six orchards were selected for the study, three in northcentral Washington, two in central Washington and one in northern Oregon, U.S.A. Each orchard was divided into a 10-acre CONV block and a 10-acre NNAI block. Pheromones were used as the primary control for codling moth in the NNAI orchards. This treatment alone was as effective as conventional azinphosmethyl sprays at two sites. High codling moth population densities at the other four sites necessitated supplementing the pheromone treatment with two other "soft" controls, mineral oil and parasitoid releases. This combination provided good control of codling moth in two orchards, but greater than 2% codling moth fruit injury was recorded at harvest in the other two NNAI orchards. Adjacent CONV orchards sustained over 1.0% fruit injury at harvest. Most of the codling moth damage in NNAI blocks was found along the orchard edge. Insufficient control of codling moth in NNAI orchards was primarily associated with the inability of selective materials to control border infestations of this pest. Leafroller populations were well controlled in all of the CONV orchards but reached damaging levels in half of the NNAI orchards. Detecting the build-up of leafroller populations in time to control them with *Bacillus thuringiensis* was difficult. Other secondary pests were generally at low levels in NNAI orchards. Natural enemies contributed to the suppression of many of these potential pests. Three species, white apple leafhopper, green apple aphid and tentiform leafminer, reached population densities that required intervention with insecticides in at least one of the CONV orchards. Detailed yield, packout and spray records have been kept for each pair of NNAI and CONV orchards and will be used to compare the economic risks and benefits of these two management programs.

22-076

PESTICIDE-TREATED TRAPS POSSESSING LONG RESIDUAL ACTIVITY FOR CONTROLLING TEPHRITID FRUIT FLIESR. J. Prokopy¹, X. P. Hu¹, B. Shasha² and M. McGuire³

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Large populations of apple maggot flies (*Rhagoletis pomonella*) have been controlled successfully in commercial apple orchards in Massachusetts (USA) by ringing perimeter-row apple trees with odor-baited sticky (Tangletrap-coated) red spheres that capture immigrating adults before they enter the orchard interior. Sticky-coated spheres are too messy to handle for the large numbers needed for widespread commercial use. Also, sticky spheres require cleaning every 1-2 weeks to maintain capturing power.

We have developed two different types of long-residual-activity pesticide-treated spheres that are equally or more effective than sticky spheres in controlling apple maggot flies and are far easier to handle. The first is a wooden or plastic sphere coated with a mixture of dimethoate (very low dose), sucrose, latex paint and shellac. The second is an inexpensive biodegradable polymeric substance shaped as a sphere, impregnated with feeding stimulant and coated with a mixture of dimethoate (very low dose) and latex paint. This technology is applicable to other forms of traps that could kill tephritid flies that feed on sucrose upon alighting on the traps.

22-078

CONFUSION AMONGST CODLING MOTH FELLOWS CONTINUES: A COMMERCIAL PERSPECTIVE ON THE IMPLEMENTATION OF CODLING MOTH MATING DISRUPTION IN NORTH AMERICAD.R. Thomson

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Mating disruption technology is increasingly being used for the control of codling moth in pome fruit production areas around the world. Some of the countries where codling moth mating technology is used commercially include the United States, Canada, Argentina, Australia, Italy and South Africa. In 1991, Isomate C Plus (Pacific Biocontrol Corp., Vancouver, Washington) became the first commercial formulation of codling moth pheromone to be registered in the United States. The total pome fruit acreage treated with Isomate C Plus has increased from approximately 1,200 hectares in 1991 to approximately 7,300 hectares in 1995. In 1996, Pacific Biocontrol expects to treat in excess of 10,000 hectares.

The successful commercialization of mating disruption technology will depend in large part on the development and implementation of a pheromone-based IPM systems approach. The objective of a pheromone-based IPM program is to effectively manage key and secondary pests in an economically, ecologically and environmentally acceptable manner. In a pheromone-based IPM system, mating disruption is the major tactic used to control the key pest(s). The subsequent reduction or elimination of insecticides for control of the key pest(s) will promote crop or orchard environments that will support higher populations of natural enemies and thus enhance the biological control of both key and secondary pests. The development of monitoring and sampling techniques in conjunction with economic thresholds are essential in order to accurately assess the biological relationships between key and secondary insects and their natural enemies and to implement supplementary controls if required. Pheromone-based IPM should be presented to growers as a long term approach and commitment to pest management. Growers should be encouraged to define yearly objectives and then identify the strategies and tactics needed to achieve those objectives.

22-077

TRAPPING-OUT, AN ALTERNATIVE METHOD TO CONTROL APPLE MAGGOT, *Rhagoletis pomonella* (WALSH) (DIPTERA, TEPHRITIDAE) IN APPLE ORCHARDSN.J. Bostanian, C. Vincent & G. Chouinard¹

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Trapping-out of apple maggot on an experimental basis has been successful for some time now. The method used is a red sphere alone or in combination with yellow panels. When panels are used, each panel is sandwiched between the two halves of the red sphere. The entire trap is coated with a thin coat of Tangle Trap and baited with a volatile lure. The traps are placed strategically at the periphery of a block (1 to 2 ha) and would intercept any maggots flying into the block. Commercially acceptable apple maggot (~ 1.5% injury) control has been obtained and data will be presented to show this as a control method.

22-079

INSECT VIRUSES AND JUVENIDS DISSEMINATED BY MEANS OF PHEROMONE TRAPS: A POTENTIAL TOOL OF INTEGRATED PEST MANAGEMENT IN ORCHARDSI. Hrdý, J. Kuldová¹, F. Kocourek², J. Beránková², O. Pultar³¹ Institute of Organic Chemistry and Biochemistry, Praha, Czech Republic² Research Institute of Plant Production, Praha, Czech Republic³ Laboratories Biola, Agricultural Cooperative, Chelčice, Czech Republic

The potential of attractant-pathogens and attractant-sterilants in the codling moth, *Cydia pomonella* and the gypsy moth, *Lymantria dispar* control was examined. Males attracted by pheromone into the trap are not killed but contaminated by the biologically active agent. Contaminated males search for females and transfer the pathogen or sterilant to them during copulation. Several designs of codlemone and disparlure baited traps were tested using the method of fluorescent paints. *C. pomonella* granulosis virus (CpGV) and *L. dispar* nucleopolyhedrosis virus were produced at laboratory scale and formulations suitable for coating inside of traps with viral preparations were developed. Field efficacy of traps containing CpGV was examined on three locations. Dissemination of the pathogens in the pest populations was detected by the Elisa test. The juvenile hormone analog W-328 (a carbamate derivative of 2-(4-hydroxybenzyl)-cyclohexanone) was selected for experiments as agent affecting reproduction capability of lepidopteran pests. Sterilizing effect of W-328 when administered to males of *C. pomonella* which were later mated with untreated females was promising (68 per cent of non-hatching eggs). The tested attractant-pathogens and attractant-sterilants are extremely species-specific and ecologically safe and may be useful in situations when the target pest occurs at low population densities.

22-080

THE USE OF PROPARGITE IN INTEGRATED PEST MANAGEMENT PROGRAMS ON APPLES

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Propargite, one of the world's leading acaricides, is used in over 60 countries on over 30 crops and 30 species of mites. It is a specific acaricide which has been successfully used in Integrated Pest Management programs (IPM) on many crops. When used at recommended rates, propargite is safe to pollinators and to beneficial predaceous and parasitic insects. In addition, predaceous mites will not be eliminated from treated areas. The use of propargite in managing the phytophagous mites, *Panonychus ulmi* and *Tetranychus* spp., on apples in the United States, Republic of South Africa and Australia will be discussed. Examples using propargite to selectively manipulate the predatory mite, *Typhlodromus* spp., and the predatory insects, *Stethorus* spp. in the orchard will be given.

22-082

POSSIBILITIES FOR THE IPM CONFORM USE OF METHIDATHION

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Methidathion is an organophosphate insecticide with a broad spectrum of activity. In spite of its initial detrimental effect on most beneficials, there are situations in several crops where it can be applied in a relatively harmless way to parasitoids and predators. A full cover spray, five to six weeks after petal fall, in citrus trees against all stages of scale insects provides up to three months of scale control. The subsequent resurgence of scales is largely controlled by reimmigrating parasitoids such as various *Aphytis* species. On pome fruit the product can be applied in a non-disruptive way to beneficial arthropods either as a dormant or delayed dormant spray. For this purpose, methidathion can be mixed with oil. This is common practice for early pest control. Predators and parasitoids tend to appear later than pests and, therefore, have a better chance to survive a methidathion spray.

A good selectivity of a compound against predatory mites is a basic requirement for its use in an integrated system on pome fruit and grapes. Methidathion-resistant strains of *Amblyseius andersoni* have been detected in the field. This resistance was built up by continued application of other organophosphates. A better survival of predatory mites in grapes could be achieved by directing the spray mist selectively to the lower parts of the plants. The survival rate of predatory mites in the upper untreated part with no or few grapes was much higher. From there the predators could reinvade the inferior treated part within a month.

22-081

EFFECTS OF PYRETHROID INSECTICIDE RESIDUES PRESENT ON FRUIT TREE BARK IN CALIFORNIA ORCHARDS

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Some California almond and stone fruit growers are choosing to use pyrethroid insecticides - either permethrin or esfenvalerate - in the dormant season or during the growing season as alternatives to organophosphate insecticides for control of the peach twig borer (*Anarsia lineatella*), scales, and other insect pests. However, it is not known if they can be used during the dormant season without inducing secondary pest problems, or how long their residues will persist on twigs.

Both permethrin and esfenvalerate were effective in controlling overwintering peach twig borers in the field trials. Results also suggested that the pyrethroid dormant sprays may affect generalist predators, but the impact of this on pest populations could not be determined.

Our laboratory studies indicated that residues of both pyrethroids are almost exclusively associated with the bark, and do not penetrate into the wood. Residues were somewhat more concentrated on node (bud) parts of twigs than on internode areas. If greater concentration of residue is present in the node (bud) parts, predator mites (which overwinter in this area) could have greater exposure to the residue. Pyrethroid extraction from twigs required an organic solvent, and residue could not be efficiently removed by washing twigs sampled from the orchards with either water or water plus a surfactant. From this observation we might assume that rainfall would not remove significant amounts of pyrethroid residue from twigs.

A lab bioassay indicated the amount of residues present on twigs were sufficient to kill the predatory mite *Galandromus* (= *Typhlodromus*) *occidentalis* placed on the bark for as much as 7 months after application of both permethrin and esfenvalerate. Since predatory mites often overwinter on the bark of trees, these results indicate that pyrethroid residues could affect overwintering predator populations.

22-083

Contribution to the control of *Sphaerolecanium prunastri* (Fonsc) on cherry trees in N. Greece.

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The bioecology of the scale insect [*Sphaerolecanium prunastri* Fonscolombe (Hemiptera: Coccidae)] was studied from 1993 to 1995 and an effort was made for its control, based on the use of Insect Growth Regulators (I G R.) and winter oils . The results indicate that *S. prunastri* completes only one generation per year. Also, it was found and identified the endoparasite *Coccophagus lycimnia* (Hymenoptera. Aphelinidae) and the predators *Chilocorus bipustulatus* (Coleoptera Coccinellidae) and *Exochomus quadripustulatus* (Coleoptera Coccinellidae). The two years field experiments for controlling the scale revealed that a winter application of paraffinic oil, mineral oil or potassium salt of fatty acids followed by an application in April (I I to I I I nymphal stage) or in July (stage of crawlers) of fenoxycarb, buprofezin and diofenolan gave successful control of the target scale insect and reduced only slightly the parasitization by the endoparasite *C. lycimnia*. However, methidathion which was used in chemical control had a detrimental effect to the endoparasite.

22-084

THE TRENDS OF DIAMONDBACK MOTH PARASITES ON CABAGGE IN RELATION TO DIFFERENT MIXED CROPS. Khuat Dang Long (Institute of Ecology and Biological Resources , National Centre for Natural Sciences and Technology , Nghia Do-Tu Liem-Ha Noi,Viet Nam)

Diamonback moth (DBM), *Plutella xylostella* (L.) is the most important pest on cabbage in Northern Vietnam . The population fluctuations and abundance of the pest were investigated weekly on cabbage (*Brassica oleracea* L. . *B. gongilodes* L.) from November to March of two years 1992-94 . The investigations carried out in two localities of Red River Delta showed that of two important parasites reared from DBM , the pest pupae parasitized by the pupal parasite *Phaogenes* sp. (*Ichneumonidae*) was very low (maximum 3.3%) in comparison with the sole larval parasite *Apanteles* (= *Cotesia*) *plutellae* Kurdj. (*Braconidae*) (average 30.8%) .

It was found that cabbage fields adjacent to maize and rice fields often have significantly lower parasitized pest population by *A. plutellae* than those on cabbage intermixed with different crops of *Solanaceae* ; *Fabaceae* groups and some other decorative flowers (*Asteraceae*) . for the maximal proportion of parasitized pest larvae was less than 25 % (average 13.1 %) on cabbage grown alone compared to percentage of parasitism up to 40 % (average 17.7 %) on the mixed planting cabbage with different crops .

In addition , cabbage fields of both above localities often treated by some pesticides as Cartap (PADAN 95BHN) , Phethoate+ Enthofenprox (CIDI M) but it was observed that peak parasitoid populations usually occurred in the middle and towards the end of the crop .

22-086

DISPERSION PATTERNS OF A MEDFLY POPULATION IN AN HETEROGENEOUS AGRICULTURAL LANDSCAPE D. Nestel, N. Israely¹, U. Kitron², B. Yuval¹

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During the last two years we have monitored, with Trimedlure traps, the medfly population build-up and spatial dispersion in an heterogeneous agricultural landscape (2X4 Km) located in the Judean Hills, Israel. The landscape is characterized by patches of different deciduous orchards, typical Mediterranean and forest vegetation and a residential area with home gardens. Adult medflies were not trapped during the winter months, suggesting that they hibernate in this elevation and latitude. During the spring, as well as late autumn, populations were higher in apple orchards. During the summer, the medfly population increases. The spatial dispersion of the medfly in the area is closely related to the seasonal availability of ripe fruit. Abandoned fig trees, which are scattered all around the area, serve as an important source of flies, and as a refugia during the intensive application of insecticides in commercial orchards. Home gardens were also found to contribute to the early build-up of the population and as refugia from pesticide applications.

22-085

PARTITIONING LEAFHOPPER (*ERYTHRONEURA* SPP.) MORTALITY IN CALIFORNIA GRAPE VINEYARDS: INFLUENCE OF GROUND COVERS, PARASITIDS AND SPIDERS (ARANEAE) M. J. Costello, K. M. Daane¹
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Experiments were conducted in table-, raisin- and winegrape vineyards in the San Joaquin Valley of California to better understand the role of cover cropping, parasitoids and predators as mortality agents of leafhoppers (*Erythroneura variabilis* and *Erythroneura elegantula*) on grapes. Ground covers were a combination of cool season legume/grass mixtures followed by warm season resident grasses. Parasitoids are primarily *Anagrus* spp., which attack leafhopper eggs; spiders (Araneae) comprised over 90% of the predator community.

Leafhopper density in the table grape vineyard was consistently reduced by approximately 25% in the ground cover treatment compared to clean cultivation. This we attribute to higher nymphal mortality because of greater vine stress, as indicated by higher berry sugar levels at harvest and lower vine pruning weights. Neither *Anagrus* parasitism (averaging 30%) nor spider population densities differed significantly between treatments. Compared to the grapevine canopy, spiders in the ground covers were found at low densities and were comprised of a different complex of species. This indicates that ground covers do not provide habitat that contributes to the buildup of spiders on grapevines. Spider species such as *Theridion* spp. were positively associated with leafhopper densities, whereas *Metaphidippus vitis* was negatively correlated. *Cheiracanthium inclusum* was the most commonly sampled spider. An exclusion experiment which decreased spider density on grapevines by 60% increased leafhopper density by 80%.

22-087

EFFECTS OF INTERCROPPING TOMATOES (*LYCOPERSICON ESCULENTUM* MILL) ON THE INFESTATION OF TOMATO FRUIT BORER, *HELI COVERPA ARMIGERA* HUBNER

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Studies were conducted to assess the effects of intercropping various vegetables in tomatoes on the infestation of tomato fruit borer, *Helicoverpa armigera* Hubner, at KRC College horticultural farm during kharif season 1995. No insecticides were used during the course of experiment. Highest infestation of tomato fruit borer (5.6 percent) was noticed in tomatoes intercropped with snap beans (*Phaseolus vulgaris* L.). Lowest infestation (3.4 per cent) was observed in tomatoes intercropped with radishes (*Raphanus sativus* L.). The fruit borer infestation levels in tomatoes grown alone, tomatoes intercropped with coriander and onion was 4.5%, 4.2% and 4.7% respectively. Greatest reduction in marketable yields of tomatoes was observed in tomatoes intercropped with snap beans followed by tomatoes intercropped with onions. Highest marketable yields was observed in tomatoes intercropped with radishes.

22-088

PREVENTATIVE PEST MANAGEMENT OF *HALOTYDEUS DESTRUCTOR* (TUCKER) AND OTHER EARTH MITES (ACARINA: PENTHALEIDAE) IN AUSTRALIAN RAPESEED

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Earth mites (*H. destructor*) and *Penthaleus major* (Dugès) consistently threaten the successful germination and establishment of rapeseed (canola; *Brassica napus*) throughout temperate Australia. The crop is mostly used in rotations and has become one of the most profitable dry-land crops grown. Accordingly, farmers are inclined towards the use of prophylactic insecticide sprays, often applied before germination. The urgency to provide effective alternatives has increased with the recent advent of insecticide resistance in *H. destructor*. There are now good prospects for the use of cultural control to minimise mite impact, through the use of a non-host crop in the previous season, and for the development of resistant varieties.

H. destructor has a wide (apparent) host range, but surprisingly, many of the cereal, pulse and oilseed crops which are known to be susceptible to mite damage are not, in fact, effective hosts. In the laboratory, mite survival and fecundity of these plants was very low. In the field, the pulse crops lupins and chickpeas resulted in extremely low mite populations by the end of the season. Hence these non-host crops may be used in rotation with rapeseed to minimise *H. destructor* populations. The constraints with this approach are that the mites may reproduce on crop weeds or in the microflora on the crop floor; or invade the subsequent rapeseed crop from the borders. A management strategy is proposed.

Despite the relatively narrow gene pool available in the *B. napus* germplasm bank, c. 10% of the lines screened showed some resistance to mite damage when placed under intense, non-choice tests. Some of these lines were derived from seed with low total glucosinolate content. The mode of resistance is being investigated but aliphatic glucosinolates do not appear to have a role.

22-090

RELATIVE SUSCEPTIBILITY OF THREE GRAPEVINE VARIETIES TO INFESTATION WITH *MACONELLOCoccus HIRSUTUS* (GREEN), (HOMOPTERA PSEUDOCOCCIDAE).

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Eight morpho-histological characters were used to evaluate the relative susceptibility of three grapevine varieties; moscati, romi and banati to infestation with *Maconellococcus hirsutus* (Green). The four morphological characters were, height of the plant, number & length of internodes, and girth of the stem. The four histological characters were the dimensions of upper and lower epidermis, palisade tissues and spongy tissues of the leaves.

According to the morphological characters of the stem of grapevine, results showed that moscati variety was the most tolerant to infestation, while romi variety was the most susceptible.

According to the histological characters of the leaves and morpho-histological change together, banati variety was the most susceptible followed by romi variety, while moscati variety was the most tolerant to infestation. Therefore it is recommended, that moscati variety be cultivated in the newly reclaimed areas of Egypt.

22-089

A SYSTEM FOR INFECTING THE SWEETPOTATO WEEVIL, *CYLAS FORMICARIUS* (COLEOPTERA:CURCULIONIDAE) WITH THE ENTOMOPATHOGENIC FUNGI, *BEAUVERIA BASSIANA* USING AN IMPROVED SEX PHEROMONE TRAP IN THE FIELD

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Four strains *Beauveria bassiana* were isolated from sweet potato field Okinawa, Japan. Among them the "Ilatuhiko" strain, separated from the sweet potato weevils (SPWs) collected at Yomitan village, showed the strongest infectiousness in the laboratory. When adults of both sexes were confined in a plastic cup treated with conidia of *B.bassiana*($9.3 \times 10^9/0.1g$), none of them survived. The mean survival period was 8.1 days in the males and 10.5 days in the females, respectively.

The system was consist of a specially designed sex pheromone trap and a holed bottle with the fungus(0.5g), in which attracted male SPWs were infected and went out again. The highest killing rate of males was 96.2% on 21 days after treatment and that of females was 24.0% on 36days in the sweet potato field. Ratio of carrier adults was male:57.9% and female:31.6%. In the treated field, the sex ratio of the SPW population was greatly skewed to the females. Females seemed to be infected through mating with carrier males.

The advantage of the system are:

- 1)High efficiency(attracted males are self-infected with the fungus)
- 2)Species specificity(using sex pheromone)
- 3)The fungus are easy to mass produce
- 4)Low cost compared with the conventional field sprays
- 5)Once attracted and infected males are expected to go back to the habitat, which accelerates dispersal of the fungus.

22-091

EVALUATION OF RESISTANCE TO ALFALFA WEEVIL, *HYPERA POSTICA* (GYLL.) (COLEOPTERA: CURCULIONIDAE) IN NINE ALFALFA VARIETIES

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Nine alfalfa varieties namely Ramandi, Codi, Bami, Yazdi, Ranger, Hamadani, Simerchenkaya, Maopa and Nikshahri were evaluated in terms of resistance to alfalfa weevil in a complete randomized block with 4 replications. This experiment was repeated for 3 years.

Results showed that there was a rather similar trend in population density associated with adult, larva and pupa number on different varieties. Some varieties showed differences related to biological cycles of alfalfa weevil and thies could be attributed to their relative resistance to this pest. However there was no differences between varieties in terms of their plant component (leaf, stem and flower), despite differences observed in the number of adult, larva and pupa on some of these varieties.

22-092

PROTEASE INHIBITORS EFFECTIVE AGAINST THE BLACK FIELD CRICKET, *TELEOGRYLLUS COMMODUS*, AND PORINA CATERPILLAR, *WISEANA CERVINATA*, PESTS OF WHITE CLOVER

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Genes encoding protease inhibitors (PIs) which bind with the major proteolytic enzymes of pest insects are being transferred to economically important crop plants to confer insect resistance.

The digestive proteases of an orthopteran and a lepidopteran pest of white clover in New Zealand were characterised and partially purified. A range of PIs were screened *in vitro* to determine their level of binding with these enzymes, and a number of PIs which demonstrated a high level of *in vitro* affinity were incorporated into artificial diet and fed to the insects. Survival, growth rate and levels of activity of digestive enzymes were measured.

Trypsin and elastase were the predominant proteases in the midgut of the black field cricket. Several PIs caused high levels of mortality and reduced growth. The two most effective were POT-2 (from potato) which inhibited both trypsin and elastase and SBTI (from soybean) which strongly inhibited elastase. Combination of POT-1 (from potato) which binds elastase and WGI-1 (from wheatgerm) which binds trypsin) caused a synergistic growth reduction.

Trypsin, chymotrypsin and carboxypeptidase A were the major proteases in porina larvae. BPTI (from bovine pancreas) which reduced digestive capacity to 28% *in vitro* and a carboxypeptidase inhibiting fraction (from potato) both reduced survival and growth rate when incorporated into diet.

Genes for POT-2, POT-1, SBTI and BPTI are now being incorporated into an elite cultivar of white clover for pest resistance.

22-094

INFLUENCE OF BIOCHEMICAL TRAITS OF RAPESEED AND MUSTARD PLANTS ON MUSTARD APHID, *LIPAPHIS ERYSIMI* (KALTENBACH) (HOMOPTERA:APHIDIDAE) INFESTATION

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The biochemical constituents (Glucosinolates, phenols, sugars and proteins) of *Brassica* cultivars, when analysed showed a differential reaction to mustard aphid infestation. Glucosinolates were found to be imparting resistance in cultivars (RLM-198, RW-29-6, RW-2-2, RH-7846, RH-7847) having high amount of it than those having less such as BSH-1, RW-15-6, RH-8113, Kranti, Krishna, RW-32-2, RK-8602, DIRA-247, PR-8805 and PR-8806. The amount of phenols from various plant parts presented an inverse trend with mustard aphid infestation. However, the high amount of protein and sugars from various plant parts of different cultivars were found to be associated with the susceptibility of the plant to the infestation by mustard aphid.

22-093

EFFECTS OF LEAF COLOR AND EPICUTICULAR WAX ON CEREAL APHID POPULATIONS IN NEAR ISOGENIC BARLEY LINES

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Host plant resistance to the natural infestation of cereal aphid species, especially *Rhopalosiphum maidis* [Fitch] and *R. padi* [L.], was assessed in four marker genotypes of near isogenic barley lines, *Hordeum vulgare* [L.]. Marker genotypes differed in leaf color (normal green and chlorina) and epicuticular wax amount and chemical composition (normal wax and glossy). They were obtained by heterozygous plants for markers and selfed in successive generations up to F₈. Aphid populations were estimated in individual F₈ plants to reveal marker genotype differences.

The aphid densities on parental line E227 (normal type) was significantly lower than that on L118 (chlorina glossy type), indicating that E227 and L118 are resistant and susceptible lines, respectively. Normal plants in color and epicuticular wax were the most resistant type among the genotypes in reaction to high density of aphid species, mainly *R. maidis*. However, the amount of gramine indole alkaloid, known as a deterrent substance, did not confer resistance to aphids in the marker genotypes due to the considerably low level.

22-095

RHIZOBACTERIA-INDUCED CROP RESISTANCE AGAINST INSECT-VECTORED DISEASE PATHOGENS OF VEGETABLES

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Specific strains of plant growth-promoting rhizobacteria (PGPR) were evaluated in greenhouse and field experiments for control of bacterial wilt of cucurbits, caused by the cucumber beetle-vectored bacterium *Erwinia tracheiphila*, and for control of cucumber mosaic virus on tomato, transmitted in a non-persistent manner by aphids. Treatment of cucumber with PGPR significantly reduced the incidence of wilt disease in plants infected by artificial inoculation and by natural beetle feeding. PGPR-treated plants exhibited reduced concentrations of the beetle feeding stimulant cucurbitacin, and beetle numbers in the field were significantly lower on PGPR-treated plants than on plants treated with insecticide. It is believed that PGPR-induced systemic resistance inhibits the accumulation of cucurbitacin in cucumber by a facultative alteration in metabolic pathway to other plant defense compounds.

In greenhouse experiments, treatment of tomato with specific PGPR strains eliminated or delayed the onset of cucumber mosaic virus symptoms. Results of ongoing experiments to determine PGPR effects on aphid feeding behavior and transmission of cucumber mosaic virus will be discussed.

22-096

**PINK BOLLWORM (LEPIDOPTERA: GELECHIIDAE)
POPULATION REDUCTION IN SHORT-SEASON COTTON
MANAGEMENT SYSTEMS: A CASE STUDY**

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A cotton management program in the Imperial Valley, CA was specifically designed to reduce pink bollworm, *Pectinophora gossypiella* (Saunders), populations in the area by optimizing the host-free period. The program established 1 March as the earliest planting date, 1 September for defoliant or plant growth regulator application and 1 November for cotton stalk destruction and plowdown. In-season gossypure-baited pink bollworm male moth activity monitoring and immature green cotton boll inspections for larval infestation were encouraged as decision making aids to determine the need for additional control action. Male pink bollworm, moth catches in gossypure-baited Lingren and delta sticky traps were significantly reduced each year from 1990 to 1994 following the initiation of the management program in 1989. Fewer larvae per cotton boll occurred in the years from 1990 to 1992. Fiber quality of commercial cotton sampled was also improved from 1989 to 1994, as compared to the 1984 to 1988 average. Male moth trap catches in gossypure baited traps placed around the perimeter of the Imperial Valley suggest that pink bollworm moth migration into the valley occurred from surrounding cotton growing areas during the growing season. Highest numbers of moths were caught in perimeter traps directionally placed toward cotton growing areas in the Mexicali, Mexico and Bard-Winterhaven and Palo Verde, CA. Cotton production, in general, was reduced during 1989 to 1994 in these areas and may have contributed partially to reduced populations in Imperial Valley, however, yield per hectare was better in 1993 and 1994 as compared to the average yield in 1984-88.

22-098

**INTEGRATED INSECT PEST MANAGEMENT STRATEGIES
FOR COTTON**

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Four integrated insect pest management (IPM) modules were tested on interspecific hybrid cotton for two seasons at Raichur, Karnataka, India. Ideal IPM module (M1-involving all ecofriendly components), practiceable module (M2-involving components easily available to farmers) and IPM module developed at Raichur (M3-involving bioagents and selective insecticides) were compared for their superiority over recommended plant protection schedule (RPP) (M4-involving only insecticides).

The incidence of sucking pests was least in M4 module and was on par with M3 and M2 module but was highest in M1 module. The activity of parasitoids and predators were noticed in all modules except M4. The number of good opened bolls and cotton yield were highest in M4, followed closely by M3 and M2 modules.

Analysis on the cost effectiveness of modules revealed higher benefits from M3 module followed by M4 and M2 and least in M1 module.

The results suggest that sole reliance on biocontrol agents cannot ensure satisfactory protection to cotton from insect pests.

22-097

**ECOFRIENDLY FORMULATIONS OF INSECTICIDES FOR
SUSTAINABLE INTEGRATED PEST MANAGEMENT IN
COTTON IN TAMIL NADU, INDIA.**

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Cotton is an important crop grown under varied ecological conditions. Three species of lepidopterous larvae viz., *Earias vittella* (F.), *Helicoverpa armigera* (Hubner) and *Pectinophora gossypiella* (Saunders) damage the squares and bolls. Indiscriminate and unscientific use of bolls. Indiscriminate and unscientific use of chemical insecticides in the last three decades has resulted in environmental problems besides control failure. In the recent past, Integrated Pest Management (IPM) is advocated to sustain the management of pests. Chemical insecticides will continue to play a major role in IPM; however, concerted efforts are made to identify safer and ecofriendly insecticides.

The usefulness of two formulations (Sandoz India - Agrochemicals) of insecticides viz., quinalphos 20 AF (Ekalux 20 AF) and an encapsulated form was studied. Field evaluation of these new formulations along with other insecticides was done with a spray fluid of 200 l/acre using a hand operated sprayer. The results revealed that i) both formulations are effective in reducing the damage caused by larvae of bollworms, ii) the formulations are not toxic to the carnivores inhabiting cotton system (parasitoids, predators such as lady bird beetles and spiders).

The three year study indicates that these new formulations are ecofriendly, fit in the IPM system in cotton particularly with small and marginal farmers.

22-099

**IPM ENTRY POINTS, CIBA'S BASIC STEPS TO COMBINE
CHEMICALS, BIOLOGICALS AND MODELLING FOR OPTIMUM
CROP PROTECTION: A CASE STUDY FROM THE VEGETABLES OF
SPAIN**

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The concept of IPM for more accurate pest management and optimum yield, rather than IPM for less pesticide, is barely touched in the public debate. The perception of IPM fitness in our chemical solutions, or lack of it, only clouds the issue - more energy should be expended now on deriving principles of adequate use for all the solutions in the IPM tool box. This applies as much for established products as for the eagerly awaited pipe-line of new chemistries.

More is required from the agrochemical industry than developing the right products. As Ciba takes its practical implementation of IPM seriously, the focus shifts beyond single-products to the whole crop ecosystem and Integrated Crop Management.

Even the farmers on our European doorstep may be caught up in spiralling pressures to intensify agricultural productivity. For example, although biologically intensive IPM systems are the standard in northern European protected cropping, the rapidly expanding new generation of producers in the Mediterranean Basin rely on chemically intensive crop protection methods in the absence of viable alternatives. Yet, Industry has a clear rôle in promoting the shift to IPM systems which rely on the right balance of available technologies. Identification and exploitation of the IPM entry points is the most effective way to initiate the process.

In this paper we examine a practical example, developed in the farmers' fields of Spain. In a step-by-step process, current Crop Protection inputs have been rationalised in methods that return tangible benefits to farmers; promote the shift to judicious and selective chemical inputs; promote the re-invasion, and intensify the impact, of native natural enemies. Finally, the ground has been prepared for true IPM systems which combine full product ranges of chemical and biological solutions.

22-100

DISTRIBUTION and MANAGEMENT of *FRANKLINIELLA OCCIDENTALIS* (Pergande) IN NEW SOUTH WALES AUSTRALIAG. Herron, V. Rajakulendran, L. Tesoriero A. Cliff

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Frankliniella occidentalis (Pergande) was first found in New South Wales in the Sydney basin (-33° 41', 150° 52'; altitude 19-170 m) during October 1993. It is now well established within the Sydney basin and is present on some properties outside this area. The thrips does not appear to migrate well unaided; most new infestations were associated with the movement of infested material. In the Sydney basin *F. occidentalis* is a pest of both field grown and glasshouse vegetables including lettuce, capsicums, strawberries and Lebanese cucumbers. In contrast to other countries, breeding populations have been found in field lettuce in mid-winter.

The proposed control strategy relies on four factors: 1) restrict the movement of infested material; 2) good hygiene in terms of weed control around the fields, polyhouses and glasshouses; 3) strategic use of insecticides on both weeds and the crop; 4) exploitation of native natural enemies. The most effective registered insecticide is methamidophos, which can be used on weeds and early stages of vegetables and must be sprayed consecutively as three sprays 3-5 days apart. When thrips numbers are reduced, populations can be maintained at low levels close to harvest using other, less potent chemicals with shorter withholding periods. Other insecticides with novel modes of action, including petroleum spray oils (PSOs) are being evaluated. Based on field results for the related species, *F. schultzei*, PSO's may be used as part of resistance management close to harvest to suppress survivors of earlier treatments.

22-102

EFFICACY OF A TRACTOR-PROPELLED VACUUM MACHINE FOR INSECT MANAGEMENT IN POTATO AND CELERY CROPS

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Over the course of 4 growing seasons, trials were run to evaluate the efficacy of a tractor propelled field-scale vacuum unit for use in insect pest management in potato and celery crops. The vacuum unit was designed to dislodge insects by blowing air from lateral vents onto the plants while simultaneously vacuuming from above. Typically, plots were vacuumed once a week over the course of the growing season. Efficacy was evaluated by yellow sticky traps and hand vacuuming plots before and after the field vacuum. All insects evaluated were effectively removed by the vacuum unit; typically population reductions of 50 - 65% were achieved with whiteflies, leafhoppers, and aphids. In some trials, reductions were achieved which lasted from week to week. Additionally, agromyzid leafminers were significantly reduced in number, but population reductions were temporary because of their strong flying ability. Parasitoids were also effectively removed by the vacuuming procedure but the effect was only temporary. In some cases, pest populations were lower in vacuum treated plots than in insecticide treated plots. There was no adverse effect on potato yield from soil compression.

22-101

AN INTEGRATED PEST MANAGEMENT PROGRAMME FOR CONTROLLING THE LEAFHOPPER *EMPOASCA DECIPiens* (PAOLI) (HOMOPTERA: CICADELLIDAE) ON SUMMER VEGETABLES IN EGYPT

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On the basis of ecological studies on the leafhopper, *Empoasca decipiens* (Paoli) attacking the vegetables in Egypt, an IPM for controlling it was designed as follows.

1-The highest peak of abundance for the pest population taking place in the last week of May suggested the usage of the chemical control (Tamaron 60% at rate 0.2%) to kill the majority of the pest population. 2-Since the squash plants harbour the highest population compared with vegetables cultivated early in March, and the green-pepper plants similarly harbour the highest population compared with those cultivated lately in mid-June, yet the chemical control should be directed mainly to the both plantations. 3-Regarding the vertical movement abundantly towards the middle parts of the plants at between 10 a.m. and 2 p.m. in May for the early plantations and at between 12 m. and 4 p.m. in August for the late ones, hence the both time periods must be taken properly into consideration when chemical control applied. 4-If the chemical control of May realized the good reduction in the population the late cultivated plants would escape from a severe infestation, if not, the release of the predator, *Coccinella undecimpunctata* Reiche which flourishes during August and July would effect the building up of the pest population. 5-Two additional bio-insecticidal applications, e.g. *Bacillus thuringiensis* Berl. at rate of 250 g./fedden, during the last week of August and in mid-September, respectively will check the weakened remaining individuals of this pest.

22-103

The efficacy of *Aphidius colemani* (Hymenoptera: Aphididae) in combination with an IGR to manage green peach aphid (Homoptera: Aphididae) on chrysanthemumsD. L. Olson, R. D. Oetting

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The Green peach aphid (GPA), *Myzus persicae* (Sulzer), is a highly polyphagous insect that has become an economically important pest of several floricultural crops throughout the US. Growers have relied on chemical insecticides to control this aphid. However, currently most insecticides do not control the GPA at low levels acceptable to growers. In our attempts to reduce this reliance on insecticides, we conducted a series of studies on the impact of *A. colemani* on GPA. This aphid parasitoid alone may not be sufficient to control an aphid population at a level acceptable to growers. Integrating *A. colemani* with Kinoprene, a host specific insect growth regulator (IGR), in an IPM program may be necessary to maintain an aphid population below an economic damage level. Preliminary data indicate that IGRs have little or no detrimental effects on *A. colemani*. Thus, an IPM program combining *A. colemani* with an IGR may have potential to maintain GPA at a level acceptable to growers.

22-104

Susceptibility to malathion of the rose grain aphid *Metopolophium dirhodum* and its natural enemy *Aphidius rhopalosiphii* cultured on susceptible and partially resistance winter wheat varieties.

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The intrinsic toxicity of malathion to *Metopolophium dirhodum* and its natural enemy *Aphidius rhopalosiphii* reared on susceptible (Maris Huntsman) and partially resistance (Rapier) winter wheat varieties was determined in the laboratory. Seven different dose levels of 15.625, 7.812, 3.906, 1.953, 0.976, 0.488, 0.244 ppm a.i. malathion were prepared and 0.2 µl applied by topical application to fourth instar aphids and four day old parasitoid. Control insects were treated with 2 µl of the solvent acetone. Dose-response relationships were compared between species using probit analysis and maximum likelihood procedures to determine trends of susceptibility, measured in terms of µg a.i./g body weight.

The bioassay result shows that, dose required to kill 50% aphids on Rapier was six times less than the dose required on Huntsman (i.e. $\log(LD_{50})$ for aphids on Rapier = 0.3672 while $\log(LD_{50})$ for parasitoid = 2.9022 which means to dose required to kill 50% aphid was 8 times less than the dose required to kill 50% parasitoid on Rapier). The slope for aphid on Rapier was steeper than the slope for parasitoid, which means any insecticide reduction against the aphid made by a resistant variety would improve the selectivity of pesticides in favour of the parasitoid.

22-106

INTEGRATED PEST MANAGEMENT OF SOIL INSECTS, AND DISEASES, IN HORTICULTURE

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Soil-dwelling insects can be severe pests of horticultural crops. Prophylactic treatment using soil-incorporated residual insecticides is now a less accepted control option, but fumigation is rising to take its place in industries that can bear the cost. The suppression of both pest insects and soil-borne diseases is seen as a benefit of this approach and a justification for use of such high-cost agents.

Concern with the longer-term sustainability of such broad-spectrum approaches to control of soil insect pests, and the need to develop lower cost techniques, has directed attention to implementing IPM strategies for their management. A desire to simultaneously control soil-borne diseases in some instances has raised the stakes, and the complexity, for developing IPM systems to achieve such a dual aim.

Improved knowledge of the biology and ecology of African black beetle *Heteronychus arator* (F.) and whitefringed weevil *Graphognathus leucomela* (Boheman) is helping to lessen the severity of management techniques. Unusual biological characteristics of African black beetle are leading to counterintuitive, but effective, treatment with benign insecticides long before the cropping cycle commences. The synthetic fumigant methyl isothiocyanate is highly potent against whitefringed weevil, and an approach being investigated for both insect and disease management is 'biofumigation' with rotational green manure crops that naturally produce various isothiocyanates toxic to both insects and diseases.

22-105

SUITABILITY OF SILAFLUOFEN (MR. JOKER®) FOR INTEGRATED PEST MANAGEMENT (IPM) IN RICE

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Research on the selective toxicity between key insect pests and their key natural enemies is advisable for the wise use of insecticides, and it is one way to fully realise pesticide management and integrated pest management (Saito et al. 1991). The sensitivity (LC_{50} -value) of the brown planthopper, *Nilaparvata lugens* (imago) to silafluofen (3.2 ppm) is 40 times higher than for the beneficial wolf-spider, *Lycosa pseudoannulata* (130.2 ppm). The positive ratio of pest versus beneficial is a suitable indicator for silafluofen (MR. Joker®) to be used in IPM-programmes.

Data from silafluofen distribution studies with radiolabelled silafluofen show that after application of 100 g a. i. silafluofen EC20/ha/400 l water, all brown plant hoppers were killed but all wolf-spiders were alive. Furthermore, the uptake of silafluofen/g body weight insect is 2.2 times higher for *N.l.* in comparison to *L.p.* An assessment of the risk concerning silafluofen and *L.p.* shows a safety factor for *L.p.* per daily prey consumption nymphs of 2.5 - 4.8 and for adult *N.l.* of 3.4-5.3 respectively. It can be concluded that hazard to *L.p.* is therefore low.

Furthermore, there is a synergy of *Beauveria bassiana* and silafluofen against *N.l.* In conclusion, silafluofen does not harm the very effective predatory wolf-spider, *Lycosa pseudoannulata* and has the potential for a synergistic effect with the insect pathogen fungus *Beauveria bassiana* to *Nilaparvata lugens*. The new selective chemical silafluofen (MR. Joker®) therefore fits very well in IPM programmes, especially in rice.

22-107

MASS REARING AND QUALITY CONTROL OF LEPIDOPTERAN PEST, *SPODOPTERA LITURA*: PREREQUISITES FOR INHERITED STERILITY PRINCIPLE IN PEST SUPPRESSION

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An appropriate rearing of large number of insects and their performance at par with those of healthy wild population form the basis of effective utilization of inherited sterility principle, that might be employed with the introduction of mass reared, irradiated substerile *Spodoptera litura* moths into ecosystem having this pest upsurge. A semi-synthetic diet was established to procure viable and similar quality insects. To obtain this, the testing of various combinations of chemical ingredients, ground dry seed source (chickpea, wheat, wheatgerm or soybean), mixed with yeast in an agar base, was carried out. The chickpea based diet was found to be ideal for its further use for mass rearing in the inherited sterility technique, because the growth, development rate, survival were found to be more on chickpea diet than other diets tested. Suitability of the various diets was determined on the basis of an index (adequacy index) incorporating growth, development, fecundity and survival into 1 empirical factor. The chickpea diet's adequacy index was 1.47 after rearing 09 generations and >1.6 after 13 generations (last 4 generations fed on chickpea diet with inclusion of sinigrin as a phagostimulant). This index value is quite comparable to 1.74 as adequacy index on natural food (i.e. castor). Insects reared on the semi synthetic diet and natural diet were tested for their population dynamics. Life tables were constructed. The net reproductive rate (R_0) and intrinsic rate of increase (r_m) of *Spodoptera litura* were ascertained to be 740.23 and 0.2045 on chickpea diet, and 785.20 and 0.2129 on castor leaf, respectively. Mass reared insects on synthetic diet were also tested for mating behaviour (frequency, propensity, nocturnal rhythmicity) and sperm behaviour (production, descent rhythm, spermatophore formation, transfer, storage and competence for fertilization). Their sperm performance was compared with that of normal wild population to understand the moths' potency to be utilized in this control measure.

22-108

THE INFLUENCE OF GAMMA RADIATION ON PEST INSECTS AND USING IT FOR GENETIC CONTROL

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For the first time in Georgia there have been carried out investigations with a view to develop a genetic method based on the biological action of ionizing radiations of controlling harmful insects. On concrete subjects: Grapholitha molesta Busk., Pieris brassicae L., Lepidotarsa decemlineata Say., and on the basis of a detailed study of life cycle, fertility, polygamy of both sexes, features of food chains, range of migration, and other important biological and ecological aspects of the matter, there have been proven the possibility and advisability of using a genetic method for controlling these pests.

Optimum stage of development, the most sensitive to gamma radiation and at the same time not showing at a high level of sterilization any weakening of important physiological processes, has been recommended for each of the aforementioned species.

22-110

BUILDING SUPPORT FOR INTEGRATED PEST MANAGEMENT: THE EXPERIENCE OF THE SMALL FARMER PROGRAMME OF THE CIBA'S CROP PROTECTION DIVISION.

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As stated in its charter, the Crop Protection Division of Ciba regards Integrated Pest Management (IPM) as the most efficient crop protection method leading to sustainable agriculture. Therefore, it designs programmes for an IPM compatible use of its products and provides appropriate information, advice and training to its customers.

The Small Farmer Programme co-ordinated by the Farmer Support Team (FST) aims at improving small-scale farmers' pest management skills. It started in 1992 in 10 pilot programme areas in countries located in Latin America, Africa and Asia. The IPM strategy currently implemented in the Small Farmer Programme comprises three areas of activities: Prevention / Observation / Intervention. Some examples are presented. The support the FST provides to farmers for the extension of IPM goes beyond pesticide use, it includes other elements for instance agronomic practices.

The result of the FST activities has been an optimisation of pesticide usage among trained farmers. This IPM oriented strategy is a contribution toward Sustainable Agriculture because:

- It increases the skills of farmers in Crop Management
- It increases the quality and the quantity of the produced crops
- It reduces the environmental impact of Pest Control Activities

22-109

PERTINENCE OF SPERM STUDIES IN F-1 STERILITY TECHNIQUE : A SPECIFIC MODALITY IN MANAGEMENT OF LEPIDOPTERAN PEST, SPODOPTERA LITURA (FABR.)

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Need of F-1 Sterility Principle to be employed in the suppression of Lepidopteran pest, Spodoptera litura was felt due to limitations in direct use of Sterile Insect Technique owing to the peculiar feature of high radio-insensitivity of this moth. Evaluation of a substerilizing gamma dose range (40-200Gy) was done to ensure involvement of increased percentage of treated moths, that would be more competitive in mating, in reproductive act. This caused production of survivors in the first filial generation (i.e., F-1), and their inherited sterility (manifested due to aberrations/heterologous translocations in holokinetic chromosomes) was noticed as latent but bonus effect which could be effectively utilized in pest control. Operation of the F-1 sterility principle seems to depend upon (i) P-1 adult behaviour, (ii) P-1 sperm behaviour, (iii) F-1 progeny development, (iv) F-1 adult behaviour, (v) F-1 sperm behaviour. The sperm complement in the spermathecae of females, mated with P-1/F-1 moths, must be optimal (in terms of quality and quantity) to prove sperm competence, and the utilization of genetically altered sperms must occur for fertilization with normal ova, so as to fulfil the aim of F-1 (inherited) sterility technique, i.e. the reproductive interaction of treated insects and their progeny with the natural population of this pest moth for its eventual suppression by producing inviable zygotes. Behaviour of substerile P-1 moths influenced the F-1 progeny, and adult (P-1/F-1) behaviour contributed to sperm behaviour. Sperm behaviour was found to be an integral component for quality of insects and it acted as a decisive factor in F-1 sterility principle. Detailed studies were carried out, in irradiated P-1 insects (100Gy & 130Gy), their F-1 progeny and normal Spodoptera litura, on the formation of both types of sperm (eupyrene and apyrene), their descent rhythm (from testes to seminal vesicle and ejaculatory duplex), sperm transfer (from male to female, within female), and finally amphimixis of eupyrene spermatozoa genome with female gamete genome.

22-111

DEVELOPMENT OF COMMODITY-SPECIFIC DEFINITIONS OF I.P.M. AND THEIR USE IN COST-SHARING AND MARKETING PROGRAMS IN MASSACHUSETTS, USA

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General definitions of Integrated Pest Management (IPM) have proven to be of limited value as the basis of cost-share programs designed to encourage grower adoption of IPM. In addition, U.S. environmental and consumer groups have questioned the reported environmental benefits of IPM, given uncertainty as to which specific practices constitute an IPM system for a given crop. Further, Massachusetts farmers have indicated that one of their principal perceived benefits of IPM, is to improve public perceptions of local agriculture. Because improved perceptions could potentially increase crop prices and/or market share of consumer food dollars, farmers have requested a means to be recognized in the marketplace as IPM practitioners.

With these facts in mind, in the late 1980's, an interdisciplinary group of scientists in Massachusetts attempted to fully describe IPM systems then ready for implementation on several important crops: apples, cranberry, cole crops, potato, strawberry, and sweet corn. Growers, private IPM consultants and other agency representatives also participated in the process. IPM practices for each crop were grouped under general categories, such as Soil and Nutrient Management, Insect Management, Disease Management, etc., and point values assigned to specific practices based on their importance and difficulty to implement.

Information is presented on how these definitions, referred to as Commodity-Specific IPM Guidelines, have facilitated growth of a USDA cost-sharing program (SP-53), and have formed the basis of a first of a kind IPM marketing program known as Partners With Nature. The latter, developed in collaboration with the Mass. Department of Food and Agriculture, has generated substantial favorable publicity for participating growers, and has the potential to become regional, based on interest and support expressed by a well-known consumer advocacy group.

22-112

MANAGEMENT SAMPLING FOR LARVAE OF THE COLORADO POTATO BEETLE, *Leptinotarsa decemlineata* (SAY) (COLEOPTERA: CHRYSOMELIDAE)

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Untreated plots of 'Kennebec' and 'Superior' potatoes were sampled for Colorado potato beetle larvae at l'Assomption, Quebec, in 1982, 1986 and 1987. Beetle larvae per stalk were counted on 74 occasions for sample sizes ranging from 50 to 200 stalks. Regression techniques were used to estimate the relationship between mean and cumulative proportion of infected stalks. These were used to set up binomial sequential probability ratio test sampling plans for pest management decision making with tally numbers equal to 0, 1, ... 10. A binomial scheme defined by tally number equal to 4 is proposed for general use and corresponding plans are given, with operating characteristic and average sample number curves.

22-114

MANAGEMENT STRATEGIES FOR *IRIDOMYRMEX* SPP. (HYMENOPTERA: FORMICIDAE) IN AUSTRALIAN CITRUS GROVES.

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Ants, particularly *Iridomyrmex* spp., interfere with the biological control of honeydew-producing insects (soft scales, aphids, mealybugs and whiteflies) in Australian citrus orchards. Excluding ants from tree canopies leads to higher predator and parasitoid populations, reduced numbers of honeydew producers, and less downgrading of fruit due to sooty mould contamination.

Trunk barriers, although labour-intensive to apply and maintain, are one option for *Iridomyrmex* control. Long-term efficacy of tested barriers was highly variable. Chlorpyrifos (Super-IQ^(R) 0.9% active w/v) and lambda-cyhalothrin (Karate^(R) 0.6% active w/v) painted on trunks were relatively ineffective. Alphacypermethrin-treated AntCaps^(R) provided control for one season (7 months), whilst chlorpyrifos-impregnated ribbon barriers (14% active w/w) have, to date, excluded ants from tree canopies for 27 to 33 months.

Baiting represents a potentially more cost-effective strategy for ant control. A commercial hydramethylnon bait, Arinosu-Koroni^(R), suppressed *I.rufoniger* gp spp. for up to 75 days (10 g bait/tree, 0.88% active w/w). The effect of Arinosu-Koroni^(R) on *I.purpureus* (F.Smith) depends on nest size; 10 g bait/nest eliminated foragers for at least 91 days on small (1 sq. metre surface area) nests, but had no effect on large (4 sq. metre) nests. Studies on seasonal foraging activity indicate that baiting programs should be initiated in late September to early October in southern New South Wales.

22-113

Integrated control of important pests of pomegranate in Varamin

By

Dr. A. Mirkarimi, Aboureyhan institute of Higher Education

In an integrated control program for important pests of pomegranate, Physical, Mechanical, Chemical and biological methods is applied against important pests such as Carob moth, Aphids, spider mites, white grubs and wood and twig borers in Varamine. For this purpose decaying fruits were collected and destroyed in the winter, late dormant spray with winter oil, insecticide application in the soil for white grubs were used - Trichogramma egg parasitoid applied as biological control methods, the results will be shown with the statistical analysis film strips and photographs.

22-115

INTEGRATED PEST MANAGEMENT IN SORGHUM, *SORGHUM BICOLOR* (L.) MOENCH IN RAJASTHAN STATE.

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Sorghum is an important food and fodder crop. East and south east Rajasthan is the chief sorghum growing area. Insect pest damage is a major constraint in sorghum production. Therefore, IPM is necessary. Entries have been screened against shootfly as well as stem borers. In shootfly 'dead hearts' percentage was the main criteria while in stem borers, leaf injury, dead hearts and tunnel length were the main parameters. The entries were found promising for both the insects viz., SPV-462, SPV-673 and ICS 4B. Crop sanitation, removal of stubbles reduced the borer infestation. Sowing along with monsoon minimise shootfly infestation. In delayed sowing soil treatment with carbofuran 3G. and phorate 10 G. @ 3.0 g and 1.5 g per meter row length respectively minimise the shootfly infestation (1.67 per cent). Whorl application of carbofuran 3G. @ 8 and 10 kg/ha on 25th and 35th day after germination, respectively reduce the borer infestation (2.95 per cent). Release of egg parasitoid *Trichogramma chilonis* in combination with carbofuran + High seed rate also lowers the shootfly infestation.

22-116

CHARACTERISTICS OF ECOSYSTEM AND IPM SYSTEM ON TEA PLANTATION IN JAPAN

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Since tea plant is perennial and evergreen, most insect pests and their natural enemies inhabit in tea plantation through the year. The management of tea plantation including insecticide application is mainly conducted on the plucking surface, and the effect of the artificial management is very small against the bush below the plucking surface. Ecosystem of tea plantation is one of the most complex agro-ecosystem. The bush below the plucking surface is important as the refuge for natural enemies, and natural enemies well control the population density of insect pests infested on mature leaves even under the insecticide application. For example, *Amblyseius wormsleyi* well controls the population density of *Tetranychus kanzawai* which is one of the most important pests of tea plant. However, biological control of insect pests infested on new shoots is difficult and insecticide application is necessary for these pests. Moreover, *A. wormsleyi* developed insecticide resistance for various kinds of insecticide. Considering these characteristics of ecosystem of tea plantation, the IPM system is proposed. In the system, both natural enemies and insecticides are used complementally. Insecticides are mainly used for the pest on new shoot and in early summer, and natural enemies well control the pest on mature leaves and in other seasons.

22-118

CHANGES OF PEST AND ARTHROPOD COMMUNITY COMPOSITION AFTER INTRODUCTION OF IPM IN APPLE ORCHARDS

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Integrated pest management (IPM) was introduced at 1 ha apple (cv. Idared) orchard in central Bohemia, and compared with an orchard under classical chemical control. Phenology and population dynamics of pest and beneficial arthropods was investigated by several methods including limb jarring, sweeping, pheromone and pitfall traps, and visual counts.

Seven of 15 important pest species were more abundant (e.g. *Anthonomus pomorum*, leafminers, leafrollers), and 8 pest species were less abundant (e.g. spider mites, *Cydia pomonella*, *Hoplocampa testudinea*) in the IPM than in the classically managed orchard. Diversity of the phytophagous community and abundance of several herbivorous species (mostly indifferent) increased following introduction of IPM. The changes in abundance were specific for different taxonomic groups and varied with their trophic specialisation.

The number of pesticide applications decreased in the IPM orchard. An analysis of the inputs and yields revealed a positive economic effect of introducing IPM (compared to classical control). This appeared due to increased yield in spite of minor decrease of product quality.

22-117

VEGETABLE IPM RESOURCES AND DELIVERY FOR NORTH CAROLINA GROWERS

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Insects threaten efficient vegetable production and markets by lowering yields, reducing fruit quality, and making harvests unreliable. Vegetable grower IPM technology includes development and availability of information, efficient delivery and application, supportive research and extension that helps people put knowledge to practical use. Grower risks include cost of control, applications of unnecessary sprays and critical decision making process.

Insect identification and recognition of damage is the first step in IPM. North Carolina State University has a Plant Disease and Insect Clinic that serves grower and homeowner concerns and trains agents and students. Over 40 counties in North Carolina have received training, equipment and supplies, and have County Plant Clinics.

The Insect and Related Pest Manual Series is an excellent resource on insect stages, biology and management. Some 50 Vegetable Insect Notes offer additional training material and handouts on selected pests.

Know and Manage Pest Posters combine insects and diseases and provide an effective visual tool for quick recognition and selection of various management strategies and tactics.

Related resources include publications, On Farm Tests, site visits, slide set/scripts, videotapes, control recommendations, etc. Training centers on N.C. Agricultural Chemical School, N.C. Vegetable Expo, Annual Extension Conference, Agent Share Session/Workshop, field days and tours.

Delivery is via written word (annual reports, publications, insect notes), electronic mail, voice mail, newsgroups, Teletip, mobile phone, radio and through distance education.

22-119

STUDIES ON THE IPM OF POPLAR HIGH-YIELD AND QUICK GROWING PLANTATION

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There are five main insect pests (*Cryptorhynchus lapathi*, *Paranthrene tabaniformis*, *Saperda populnea*, *Coccus mongolicus*, *Maladera orientalis*) on poplar high-yield and quick growing plantation in Liaoning Province.

We studied their life-histories, best control time and best control insect stages by using light traps, sex pheromones, accumulated temperature analyses, etc. Forest management measures include seedling quarantine; spreading strip mixed by Cathay poplar and Black poplar groups; trimming off dead branches. Biocontrol measures include attracting adults of *Paranthrene tabaniformis* with sex pheromones and protecting and utilizing insectivorous birds. Chemical control measures include smearing insecticide rings and spots and stopping up pest hole with aluminium phosphide. Because of the best work programs have been selected and the insecticide application equipments have been improved, the work efficacy is raised for 3-4 times and the costs are reduced. The total control result is raised from 73% to 94%. The size of stand which have been controlled increased 3.6 times when compared with the untreated stand.

22-120

INTEGRATED PEST MANAGEMENT IN APPLE ORCHARDS.
EXPERIMENT IN ROMANIA

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The experiment within the IPM project was organized at the Fruit Tree Research Station, Volnesti, in a 2 ha old orchard (26 year) and in a new orchard planted in 1994 (2 ha), half with cvs Generos and Florina, resistant to apple scab.

Buckwheat (*Fagopyrum esculentum*), dill (*Anethum graveolens*), sorghum (*Sorghum bicolor*), vetch (*Vicia sp.*) and coriander (*Coriandrum sativum*) were undersown along the tree rows.

Comparison between the IPM system and chemical supervised control in the old orchard was favourable to IPM. Fruit quality was slightly superior in the chemical supervised control plot. The amount of beneficial fauna was much higher in the IPM plot. The number of sprayings was reduced in the IPM plot to 12, compared to 16 in the supervised control plot.

In the young orchard with resistant cvs superiority of IPM was demonstrated by a low number of sprayings, low amount of active ingredient per hectare (3.18 kg/ha a.i. instead of 10.64), as well as a good activity of beneficial arthropods.

22-122

COMPREHENSIVE MANAGEMENT TECHNIQUE OF TETTIGONIELLA VIRIDIS(L)
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Tettigoniella viridis(L) is very popular in Chinese farming and forestry. The host plants are amount to 39 families, 102 genus and 166 species. Among them, the gardens plants reach more than 30 species.

In Xinjiang province the pest breaks out twice every year. the hibernating hatching ova accumulated temperature is higher than 0°C reaching more than 400°C. The raging period of incubation of hibernating ova of the pest coincides with phenological phase of pear blossoms being out. The raging period of oviposition, in autumn coincides with phenological phase of oleaster ripeness being out. Interval between the mass appearance of first generation of the adult and the raging period of oviposition is about 30 days. It is worked out that Control index of the pest, to one-year and two-year poplar trees is 20-25egg lumps/0-50cm. branch 11-14 adult pests per-tree.

The pests egg-lump distribution belongs to assembly distribution.

The integrated Control measure is joint defence by agriculture and forestry. It includes Weeding, killing ova pruning branches infested with pests. Combined with selecting pestresistant tree species such as populus italica. The Categories reach to 66 species. luring adult pests to their death with black light. protecting *Allothrombium pulvinum* E. and other six species of natural enemies spraying with 2.5% Leltamethrin milk oil to Control the adult pests.

22-121

INFLUENCE OF DIFFERENT SOURCES OF
PHOSPHORUS IN MAIZE (*Zea mays* L.) ON THE *Spodoptera frugiperda* (J.E. SMITH, 1797) DEVELOPMENT.

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The influence of different sources of phosphorus in maize affecting *Spodoptera frugiperda* development was observed in Faculty of Agronomy "Manoel Carlos Gonçalves" - Espírito Santo do Pinhal - São Paulo. The cultivar Dyna 170 was grown in greenhouse conditions. The insects were fed with maize in laboratory at 26° C and 12 hours of light.

The treatments arranged in a complete randomized design with 8 replications were : A- control; B- NK; C- NPK (single superphosphate); D- NPK (triple superphosphate); E- NPK (phosphate rock); F- NPK + P (double dosage of single superphosphate); G- NPK + P (double dosage of triple superphosphate); H- NPK + P (double dosage of phosphate rock). The treatments were based upon soil analysis.

Length, weight of larva and pupa, larval and pupal period were evaluated.

The lack of fertilization and phosphate rock application induced to the least length, weight of larva and pupa and also to a minor pupal period. The larval period was the greatest with phosphate rock. The parameters evaluated were greater with single and triple superphosphate excepting to larval period.

22-123

INTERCROPPING AS A PEST MANAGEMENT STRATEGY
AGAINST CARROT FLY (*PSILA ROSAE* F.)

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Intercropping of carrots and lucerne, *Medicago littoralis* Rohne ex Loisel. was found to be effective in reducing intensity of carrot fly damage. The studies were carried out during 1988-1994. The mechanisms operating in the intercropping system were studied. The influence of natural enemies and the importance of carrot fly host-finding behaviour were studied. However, there was no correlation between the amount of predators (staphylinids, carabids and spiders) and damage level of carrot fly. In an experimental study densities of polyphagous predators were enhanced and decreased. A significant differences in damage between the monocultural and intercrop treatments was found, however, irrespective of predator density. The importance of an intercrop for host-finding behaviour was tested by measuring the number of flies in monoculture and intercrop with lucerne in field experiments arranged in three different ways. More flies were caught in the monoculture treatment than in the intercrop, when plots were placed next to each other or placed apart from each other surrounded by ley. However, no differences were found between treatments when plots were apart and surrounded by bare soil. Nevertheless damage levels were always lower in the intercrop treatment. In greenhouse experiments egg deposition by the carrot fly was lower in intercropping.

The yield decrease due to competition from the intercrop was quantified and assessed with respect to the benefits of the intercrop in terms of reduced damage from the carrot fly, soil nitrogen enhancement through N-fixation, and decreased weed germination and development.

22-124

LONG TERM EFFECT OF RATES OF INSECTICIDES AND REFUGIA UPON NATURAL ENEMIES OF SOYBEAN PESTS.

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A field experiment was conducted at CNPSo/EMBRAPA experimental station, during the 1992/93 soybean season, aiming to evaluate the impact of insecticides, with different schemes of application, on predators and parasitoids of soybean pests, along all the soybean cycle. Experimental design used was random block, with three replications, and plots measuring 20x70 m. The treatments consisted of two insecticide applications (velvetbean caterpillar / stink bug control). Insecticides and rates used were: a. *Baculovirus anticarsia* 20 g pc.ha⁻¹/endosulfan 219 g ia.ha⁻¹ + NaCl 0,5%; b. endosulfan 175 g ia.ha⁻¹/endosulfan 438 g ia.ha⁻¹; c. lambda-cyhalothrin 3.75 g ia.ha⁻¹/lambda-cyhalothrin 7.5 g ia.ha⁻¹; d. monocrotophos 80 g ia.ha⁻¹/monocrotophos 150 g ia.ha⁻¹; e. *B. anticarsia* 20 g pc.ha⁻¹/monocrotophos 150 g ia.ha⁻¹; f. *B. anticarsia* 20 g pc.ha⁻¹/monocrotophos 100 g ia.ha⁻¹ + NaCl 0,5%, applied on strips of 1.5 m spaced by 1.5 m without insecticide; g. profenophos 80 g ia.ha⁻¹/monocrotophos 100 g ia.ha⁻¹ applied on strips of 3m spaced by 3m without insecticide; and h. check. The shock method was used to evaluate the insect population, collecting dead insects fell on a ground cloth after 15 minutes. The insects collected on the samples were identified and counted on the laboratory. Statistical analysis did not indicate differences among the treatments relating to number of individuals of each major predators or parasitoids species, or even when grouped by family, order or feeding habit. The major number of Diptera and Hymenoptera specimens were consistently collected on plots where monocrotophos was sprayed.

22-126

INDIVIDUAL EVALUATION OF RICE RESISTANCE TO THE GREEN RICE LEAFHOPPER, *NEPHOTETTIX CINCTICEPS* UHLER (HEMIPTERA: DELTOCEPHALIDAE)

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Accurate and rapid technique to evaluate individual level of resistance for genetic analysis was established. Resistance of eighty B₁F₁ plants, from the cross between resistant Norin-PL6 and susceptible Toyonishiki, was evaluated by two methods: seedling test and leaf blade test for antibiosis. In the seedling test, five newly hatched nymphs were caged with one seedling at the first-leaf stage in a test tube. Nymphal survivorship and stage were recorded after 3-4 days. We could not find a clear segregation because of continuous distribution using only survivorship. However, the segregation of B₁F₁ plants was clear using two indices: nymphal survivorship and stage. In the leaf blade test, the result was similar in the seedling test using two indices. Genetic analysis of resistance using individual test and molecular markers (restriction fragment length polymorphism) indicated that the resistant genes for green rice leafhopper located on chromosome 3 and chromosome 11 in Norin-PL6.

22-125

MECHANISMS OF GROUND COVER EFFECTS ON *ERYTHRONEURA VARIABILIS* AND *ERYTHRONEURA ELEGANTULA* (HOMOPTERA: CICADELLIDAE) IN CALIFORNIA VINEYARDSR. Hanna¹, F. G. Zalom¹, L. E. Williams¹, E. A. Weber²¹Departments of Entomology and Viticulture & Enology University of California, Davis, CA USA- ²University of California Cooperative Extension, Napa, CA USA

We have conducted several large and small scale experiments to determine the effect of ground cover management on two species of leafhoppers, which are serious pests of grapevines in California vineyards. Ground covers consisted of fall-planted grass and vetch mixtures that were either plowed down in mid-spring or early summer. Leafhopper infestations were reduced substantially in some vineyards in the presence of vegetative ground cover. Generally, leafhopper numbers were lowest in the early-summer plow down, intermediate in the mid-spring plow down, and highest in the bare-ground plots. The underlying mechanisms behind these observations were site-specific. On 'Thompson Seedless' grapevines in the San Joaquin Valley, lower leafhopper densities were caused by higher densities of spiders and probably non-host interference by the ground cover. Differences in vine-nutrient and vine-water status were not important. On 'Sauvignon blanc' grapevines in the Napa Valley, lower leafhopper densities were associated with greater water and nitrogen stress. Spiders in the young 'Sauvignon blanc' vineyard were very low on all vines and were not affected by the presence of ground cover.

22-127

MECHANISM OF RESISTANCE TO THE GREEN RICE LEAFHOPPER, *NEPHOTETTIX CINCTICEPS* UHLER (HEMIPTERA: DELTOCEPHALIDAE) AND SUPPRESSION OF THE LEAFHOPPER POPULATION IN THE FIELDS IN THE PARENTAL AND BREEDING LINES OF RICE

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Green rice leafhopper (GRLH) is an important insect pest of rice in Japan. To use the resistance of rice for integrated pest management in GRLH, screening on resistance has been conducted in Japan. Several Indica varieties showed considerable resistance, while all Japanese varieties were susceptible. We tested the resistance of 4 parental lines and 5 breeding lines to the GRLH in laboratory and field conditions. The resistance genes of these lines are derived from Indica varieties. A high nymphal mortality was observed on most of the parental and breeding lines at early tillering stage. In contrast, a few breeding lines showed moderate resistance to the GRLH. The egg production was reduced to 4/10-7/10 on these breeding lines, as compared with susceptible varieties. On the other hand, all the parental and breeding lines were less preferred by the female adults. The population density also remained significantly low both in the parental and breeding lines, while the density increased in the susceptible varieties. These results suggest that the mechanism of resistance resulting in the suppression of the GRLH population in the fields is associated with non-preference and antibiosis. This indicates a possibility to use the moderate resistant breeding lines for IPM in the GRLH in Japan.

22-128

RESISTANCE OF SOME PEAR GENOTYPES TO PEAR PSYLLA PSYLLA PYRI L.

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Pear psylla (*Psylla pyri* L.) is a major pest in the plantation orchards in Yugoslavia. Insecticides have been mostly used in the control of this pest so far. The paper presents the first obtained results of the study on resistance of some pear cultivars to pear psylla.

The investigations were conducted in varietal plantings of the Fruit and Grape Research Centre, Čačak over the 1991-1994 period.

The criteria for resistance evaluation were grouped as follows: parameters of population dynamics of pear psylla and parameters of damage degree (defoliation, honeydew and sooty mould).

The tests included 20 pear cultivars. Moderate resistance was shown by three cultivars (Magness, Karamanka and Vidovača).

These cultivars should be used for breeding other resistant cultivars due to their good pomological properties.

22-130

MOLECULAR BIOLOGICAL AND GENETICAL ANALYSIS OF THE EFFECTS OF RADIATION AND RADIOISOTOPES ON INSECT STERILITY

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In the sterilization of male insect by irradiation the important function is to obtain a genetic dominant lethal mutation. Pupae of insect were treated with radiation, the organelles in the germ cell showed damage, which may be used to produce the sterile male insect. Radiation must be given before meiosis in order to produce mutant. Insects were fed on radioactive diets. Changes of the structure of the organelles in germ cell and adverse effects on fertility are examined. The chromosome became aberration will bring about the decrease of propagation ability. The damage to centrioles is associated with loss of sperm activity. The damage to mitochondria is associated with abnormal insect development. The damage to lysosome is believed to be responsible for destruction of protein. Spermatocytes were examined by the light microscope and transmission electron microscope. Proteins, amino acids, and peptides were analyzed by electrophoresis.

22-129

DETERMINATION OF ANTIBIOSIS AND ANTIXENOSIS TO *ALABAMA ARGILLACEA* (HÜBNER) AND *HELIOTHIS VIRESCENS* (F.) (LEPIDOPTERA: NOCTUIDAE) IN COTTON GENOTYPESG. Contreras¹, G. Videla¹, J. Poissón², M. Polak¹, A. Ruiz¹¹ Departamento de Entomología, Instituto Nacional de Tecnología Agropecuaria, Sáenz Peña, Chaco, Argentina² Departamento de Genética, Instituto Nacional de Tecnología Agropecuaria, Sáenz Peña, Chaco, Argentina

Ten genotypes (three cultivars and seven advance lines) were tested to determine types of resistance to *Alabama argillacea* (Hübner) and *Heliothis virescens* (F.) in both lab and semi-field conditions, in Chaco, Argentina, during 1995. Insects used in the experiments came from rearing facilities ($25 \pm 3^\circ\text{C}$, $60 \pm 10\%$ RH, 14:10 L:D).

Antibiosis was evaluated feeding neonate larvae of *A. argillacea* and *H. virescens* with cotton leaves and squares, respectively, of each genotype. Completely randomized design tests with fifteen replications were performed in laboratory. Larval weight at seven days, time to pupation, pupal weight and time to adult emergence was registered.

Antixenosis was evaluated in field conditions. Potted plants were included in 36 m² cages, where 50 mated females of either species were released for each test. Moths were allowed to oviposit overnight. Next day the number of eggs deposited on leaves, stem and bracts was registered. Tests were run during five different dates, each with 10 replications in a randomized complete block design.

Antibiosis tests showed significant differences ($P < 0.05$; $n = 3$) among genotypes. The lightest larvae were obtained from genotypes "Guazuncho 2 INTA" and "pool of advanced lines".

Significant differences ($P < 0.05$; $n = 5$) were also obtained among genotypes tested for antixenosis. The highest levels were observed in genotypes "without trichomes" and "okra leaf without trichomes".

22-131

HELIOTHIS VIRESCENS (F.) (LEPIDOPTERA: NOCTUIDAE): ANALYSIS OF MOVEMENT DATA AND ESTIMATION OF THE SCALE OF AREA-WIDE SUPPRESSION PROGRAMS

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Suppression of *Heliothis virescens* (F.) over an area large enough to appreciably delay population increase due to immigration has been attempted by release of a sterile male-producing interspecific hybrid, destruction of wild host plants, and application of a nuclear polyhedrosis virus. The results from these tests have been equivocal: due, in large part, to inadequate size of the areas treated relative to typical distances moved by *H. virescens*.

Releases of up to 1,600,000 laboratory-reared males were made near Greenville, MS, USA during eclosion of overwintering populations in each of 1982, 1983, 1993, and 1994. Males were recaptured in up to 120 pheromone traps over an area of 4,000 km². Cumulative trap catch was fitted to a model that assumed a constant diffusion coefficient and rate of mortality. Estimates of median distance moved are 11, 8, 23, and 16 km, respectively.

In 1994, blanket treatment with nuclear polyhedrosis virus of an area 32 km in diameter near Greenville, MS, USA resulted in a 59% reduction in survival of the offspring of overwintering *H. virescens*. Movement into this area of suppression was simulated by a computer program that assumed movement of females is the same as that observed for males, movement is identical in different generations, and population growth rate is density independent. For a median distance moved of 16 km, suppression of the next larval generation was estimated to range from 35% to 18% at the middle and edge of the suppression area, respectively. The level of suppression in the following generation dropped to 23-15%. Given the sample sizes used, these levels of suppression were below statistically detectable levels; and, indeed, treatment effects were not significant.

22-132

LOW COST MEDIA OF THE MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (WIED.) (DIPTERA: TEPHRITIDAE) FOR USE IN AN INTEGRATED MANAGEMENT SYSTEM

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Low cost larval media were evaluated for rearing the genetic sexing strain GSWP-1-62 (white pupa) of the Mediterranean fruit fly, *Ceratitis capitata* (WIED.) (Diptera: Tephritidae), as part of a male sterile release program in an experimental citrus orchard in Greece. The media were made up of low cost and locally available ingredients.

The media proved to be equivalent or better when compared to a control which was routinely used for mass rearing of this insect. The best medium gave pupal number higher than the control and all other parameters recorded equivalent to the control. Thus number of pupae/g for this medium was 18.0 compared to 10.3 for the control, while hatchability of eggs, pupal weight and adult emergence was 82.7 %, 8.1 mg/pupa and 95.2 % for the experimental medium and 79.7 %, 8.5 mg/pupa and 94.3 % for the control, respectively. The effect of certain other ingredients was also evaluated.

22-134

ATTRACTIVE CAPACITY OF AGGREGATING PHEROMONE TO ADULTS OF THE COTTON BOLL WEEVIL, *Anthonomus grandis* BOHEMAN (COLEOPTERA, CURCULIONIDAE) DURING BETWEEN-SEASON PERIOD

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The cotton Boll Weevil, *Anthonomus grandis*, was detected in São Paulo State, Brazil, in 1983 (Habib, Fernandes, 1983) and now is considered as a key pest in cotton fields in this country. The present study was undertaken to investigate the attractiveness of boll weevil adults to its aggregating pheromone (grandlure) under winter field conditions.

At the end of the cotton season, adult populations disperse looking for alternative food and reproductive resources and refuges to spend the between-season period. Attraction and kill could be considered as a benefit strategy during such a period. Two experimental fields, at Casa Branca City, State of São Paulo, were utilized. For each one, three areas (300 m² each) were established near forests or orchard, with 500 m distance between them, to receive pheromone applications. Each area was divided in three sub-areas (100 m² each). The distance between the 1st. sub-area and the refuge was 20 m; while the distance between the 3 sub-areas was only 10 m. The pheromone capillaries were distributed homogeneously (2.5 g per m²). In addition to the pre-application counting, 5 evaluations were realized after treatment in each sub-area. 10 randomized samples (1m² each) was considered for each sub-area. Boll weevil adult response to pheromone was immediate, and prolonged up to 14 days. The highest level of attractiveness, however, was observed 24 hours after applications. Chemical control in small plots, can be recommended 24 hours after pheromone applications as a between season strategy for the control of *A. grandis* adults.

22-133

MEDITERRANEAN FRUIT FLY ERADICATED FROM CHILE: ONE MORE SUCCESSFUL APPLICATION OF THE STERILE INSECT TECHNIQUE

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A Sterile Insect Technique (SIT) Mediterranean fruit fly eradication project in Chile culminated in the eradication of this pest from the country, with benefits to the economy estimated at US\$ 500 million per year. In a ceremony presided over by the former President of Chile, the country was officially declared Mediterranean fruit fly-free on December 1995.

Although the southern and central regions of Chile were already free of fruit flies and the country had developed a very successful fruit export industry, its produce was still being restricted from certain international export markets because of fear of outbreaks originating from the presence of medfly in the Arica region in northern Chile. After a decade of unsuccessful attempts to eradicate the fly using insecticides in the Arica region, the Chilean Agricultural Service, with the technical and financial support from the IAEA, initiated a Sterile Insect Technique eradication programme against this pest. As a consequence, a medfly mass rearing facility with a production capacity of ca. 60 million sterile flies per week was completed in 1993, when sterile fly releases were initiated. No wild medflies have been detected in the Arica region since the first half of 1995. In addition, the pest has already been suppressed in Tacna, the southern-most valley of Peru.

Future collaboration within an expanded FAO/IAEA bi-national Chile-Peru project foresees enlarging the eradication and control activities to other fruit-producing valleys in southern Peru.

22-135

CONTROL OF *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) (LEPIDOPTERA: GELECHIIDAE) USING TWO PHEROMONE MATING DISRUPTION METHODS

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Pink bollworm (pbw), *Pectinophora gossypiella* (Saunders) is a relatively serious pest of cotton toward the end of the season in Argentina. Chemical control is avoided due to insecticide resistance management program for other pests, such as Heliothinae. Therefore, emphasis is devoted to evaluate more specific type of controls. Pbw pheromone (Hexadecadienyl Acetate) is used throughout the cotton area of Argentina to bait monitoring traps only. Rings containing pbw pheromone (Selibate PBW-rings[®]) and sprayable pbw pheromone formulations (Check Mate[®] and No Mate[®]) were evaluated as mating disrupters during 1995 and 1996 (in progress).

Field tests were performed. Rings were evaluated in an 8 ha plot during 1995; the other two formulations were tested in large areas (more than 500 ha each), during 1995 and 1996. When significant differences ($P < 0.05$) in captured pbw moths were detected between treatments (with and without pheromone), higher numbers were found in control plots. No significant differences between treatments were detected for infestation of fruit forms. Seed index was significantly lower in pheromone treated plots. More lint resistance was observed, although not always significant, in plots with pheromone.

For the given experimental conditions, results are considered promising in favor of a reduction of the pest in pheromone treated plots. Practicality of both methods, and also 1996 results are discussed.

22-136

EFFICACY EVALUATION OF *BACILLUS THURINGIENSIS* AND NEEM SEED OIL, *AZADIRACTA INDICA* ON POTATO TUBER-MOTH IN THE FIELD AND DURING STORAGE

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A new approach was used for evaluation of the efficacy of *Bacillus thuringiensis* and pure neem seed oil on the potato tuber-moth, *Phthorimaea operculella* in the field and store. Both the two materials were found equally effective on the potato tuber-moth infestation. The infestation percentage could be significantly reduced by frequent application of both in the field up to harvest and also during the storage. No clear synergistic effect could be observed upon application of the two preparations simultaneously. The results obtained suggest their possible use as substitutes for hazardous chemical insecticides in combating the pest both in the field and during storage.

22-137

INTEGRATING *BACILLUS THURINGIENSIS* INTO AN IPM PROGRAM IN CELERY

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Economic analyses were conducted on celery plantings in 1992 and 1993 at a field station which compared the benefit of current 'chemical standard' pesticide practices to an IPM program based on a registered *B. thuringiensis* preparation and an untreated control. The chemical standard treatment consisted of 9 applications of methomyl and permethrin. The IPM program used 3 or 4 applications of *B. thuringiensis* as needed. The impact of each pesticide use program on insect populations, fruit damage, yield, crop value, cost of control, and net profit was determined. Both the chemical standard and IPM treatments reduced pest populations and damage, resulting in better yield and net profits as compared to the control treatment. The reduced input costs of the IPM program resulted in better return in both years. In 1992, net profits were higher by about \$200/ha. In 1993, all treatments lost money, but the IPM program lost approximately \$60 less per ha than the chemical standard treatment. In 1995, a large scale, replicated trial was conducted with a commercial celery grower. The IPM program proved significantly more economical. Fewer applications of less toxic pesticides were required to produce an equivalent amount of product. The economic results from the IPM program were conservative as some substantial benefits were not included in the analyses. These include a reduction in development of pesticide resistance, reduced soil compaction, less potential for damage to the environment, and less possibility of human health concerns.

22-138

BIOLOGICAL AND INTEGRATED CONTROL PROGRAM AGAINST INSECT ATTACK ON BELL PEPPERS GROWN IN GREENHOUSE
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In order to develop a fruit growing greenhouse system with a minimum input of pesticide, five different sweet pepper growing systems were set up, compared and managed.

One system was a biological greenhouse system, the second system was an integrated one, the third system was an integrated system in which were used biopesticides (such as *Bacillus thuringiensis* Berliner sub sp. *kurstaki*) instead of chemicals, the fourth was a system where only chemicals were applied and the last one was an untreated one. The experimental design was a complete randomized block replicated four times. The target pests were *Frankliniella occidentalis* (Pergande) and *Ostrinia nubilalis* (Hb.). They were monitored by observing flowers and blue traps (thrips), by using pheromone traps (moths) and by evaluating the fruit damage (both). Yield and earliness were noticed.

The best control of *F. occidentalis* was obtained for the biological and integrated systems where *Orius laevigatus* (Fieber) was released four times from June 1st to July 6th, 1995. Less damage to fruits was observed for the same systems too. With regard to *O. nubilalis* control, the best results were registered only by the systems where integrated control was applied by using hexaflumuron or *B. thuringiensis* *kurstaki*.

The highest yield was obtained by the systems including integrated control and only chemical applications. The best earliness was performed by biological and untreated systems. Lack of chemicals induced the plants to earliness.

If invited speaker, indicate title of session and name(s) of organizer(s):

22-139

CONTROL OF *CACOPSYLLA PYRI* (L.) (HEMIPTERA: PSYLLIDAE) BY JUVENILE HORMONE ANALOGUES

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The commercial juvenoids, methoprene and hydroprene as well as twelve new juvenoids were tested on the populations of *Cacopsylla pyri* (L.) (Hemiptera: Psyllidae) and on the predatory bug, *Anthocoris nemoralis* (Fabricius) (Hemiptera: Anthocoridae) by the indirect contact application.

It was found that even low doses of the active juvenoids evoked in *C. pyri* the malformations of wings and external genitalia which disabled them from flying and copulation. The period of sensitivity to juvenoids comprised in *C. pyri* the first 4 days of the last instar nymphs, in *A. nemoralis* only the first 24 hr of the last instar nymphs. Sensitivity of *A. nemoralis* to the tested juvenoids is very low. Thus juvenoids could be a useful tool for the control of psyllids without endangering their natural regulators, the bugs family Anthocoridae.

22-140

APPLICATION AND IMPORTANCE OF INSECTPATHOGENIC VIRUSES IN RESPECT TO THE INTEGRATED PEST MANAGEMENT (IPM)

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There is given a survey about possibilities of using insectpathogenic viruses as biological pesticides in frame of Integrated Pest Management Systems (IPM). Results of control experiments with insectpathogenic viruses against *Mamestra brassicae* L., *Agrotis segetum* SCHIFF. and *Cydia nigricana* Steph., respectively, under greenhouse and field conditions are interpreted. The merits and disadvantages of insectpathogenic viruses are discussed.

22-141

LONG TERM EFFECT OF INSECTICIDES UPON NATURAL ENEMIES OF SOYBEAN PESTS.

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A field experiment was conducted at Sertãozinho county (Paraná-Brazil), during the 1993/94 soybean season, aiming to evaluate the impact of insecticides on predators and parasitoids of soybean pests, along all the soybean cycle. Experimental design used was random block, with three replications, and plots measuring 100x100 m. The treatments consisted of two insecticide applications (velvetbean caterpillar / stink bug control). Insecticides and rates used were: a. Endosulfan 210 g ia.ha⁻¹/endosulfan 525 g ia.ha⁻¹; b. lambda-cyhalothrin 4.5 g ia.ha⁻¹/lambda-cyhalothrin 9 g ia.ha⁻¹; c. monocrotophos 96 g ia.ha⁻¹/monocrotophos 180 g ia.ha⁻¹; d. *Baculovirus anticarsia* 24 g pc.ha⁻¹/monocrotophos 120 g ia.ha⁻¹ + NaCl 0,5%; and e.check. The shock method was used to evaluate the insect population, which consisted on the application of 2% of mevinphos, a potent and broad spectrum insecticide, collecting the dead insects fell on a ground cloth after 15 minutes. The insects collected on the samples were identified and counted on the laboratory. Statistical analysis indicated that lambda-cyhalothrin was initially more toxic to predators, and that none of the treatments affected the parasitoids species present on the field, or the parasitism index on caterpillars or on stink bug eggs. Larval mortality by entomopathogenous was larger on plots where insecticides were applied, probably due to sub-lethal intake rates, which stressed the surviving larvae, and depressed its enzymatic detoxification system.

22-142

SELECTIVITY OF INSECTICIDES UPON NATURAL ENEMIES OF SOYBEAN PESTS, EVALUATED SEASON-LONG.

I. C. Corso, D. L. Gazzoni

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During the 1994/95 soybean season, a field trial was conducted at Sertãozinho county (Paraná-Brazil), aiming to evaluate the impact of insecticides on predators and parasitoids of soybean pests, along all the soybean cycle. Experimental design used was random block, with three replications, and plots measuring 100x100 m. The treatments consisted of two insecticide applications (velvetbean caterpillar / stink bug control). Insecticides and rates used were: a. Endosulfan 175 g ia.ha⁻¹/endosulfan 437.5 g ia.ha⁻¹; b. lambda-cyhalothrin 3.75 g ia.ha⁻¹/lambda-cyhalothrin 7.5 g ia.ha⁻¹; c. monocrotophos 80 g ia.ha⁻¹/monocrotophos 150 g ia.ha⁻¹; d. *Baculovirus anticarsia* 24 g pc.ha⁻¹/monocrotophos 100 g ia.ha⁻¹ + NaCl 0,5%; and e.check. The shock method was used to evaluate the insect population, which consisted on the application of 2% of mevinphos, a potent and broad spectrum insecticide, collecting the dead insects fell on a ground cloth after 15 minutes. The insects collected on the samples were identified and counted on the laboratory. Statistical analysis did not indicate differences among the treatments on any of the sampling dates, considering the predators or parasitoids population, or the parasitism indexes. Although not statistically different, the lower predators population was found on treatments of endosulfan, lambda-cyhalothrin and monocrotophos, when applied twice, for the control of velvetbean caterpillar and stink bugs.

22-143

PETROLEUM SPRAY OIL: SPRAY VOLUME, OIL CONCENTRATION AND INSECTICIDAL ACTIVITY

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Since the development of low-volume and ultra-low-volume sprayers in the 1970s there has been a tendency to introduce this technology into horticultural crops. Its main advantages are reduced machinery and labour costs, and less wastage of active ingredient. This is achieved by optimising coverage using ≤ 200 μ m droplets.

In field and laboratory studies we have demonstrated that low-volume spraying is not appropriate for the application of petroleum spray oils (PSOs). When oil deposit is held constant there is a significant increase in PSO efficacy as spray volume is increased. PSOs require minimum amounts of diluent to be effective. We suggest that thorough wetting of sprayed surfaces and droplet coalescence are required for their effective use. For PSOs, run-off should not be considered waste.

22-144

INTEGRATED PEST MANAGEMENT OF EGGPLANT ARTHROPODS WITH SELECTIVE PESTICIDES: A PLAN TO CONTROL SPIDER MITES, *Tetranychus* spp., RESURGENCE.

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Eggplant in experimental plot were continuously sprayed with the insecticide permethrin, milbemectin, and fenbutatin oxide to evaluate their respective side-effects. Effect of population of pests and of their natural enemies were assessed. The result revealed the importance of natural enemies such a anthocorid bugs, *Orius* spp. Application of permethrin may cause resurgence of *Aphis gossypii* and *Thrips palmi* population because of (1) the development of resistance by the pests and (2) nonselective killing of the predators.

The author therefor attempted to develop methods of control of pests that would not affect substantially natural enemies. Respective treatment with granules of Asetamiprid, Nitenpyram and Imidacloprid at the time of planting enabled to control *A. gossypii* and *T. palmi*. But these pesticides caused resurgence of *Tetranychus* spp.

Milbemectin and fenbutatin oxide which exerts minimaized adverse effect on *Orius* spp. when used in combination with the granule of Imidacloprid, Asetamiprid or Nitenpyram, maximized these granule's advantage while minimizing those disadvantages.

22-146

KNOWLEDGE-BASED DECISION SUPPORT SYSTEM FOR MANAGEMENT OF WESTERN AND NORTHERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE)

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Research on the biology, ecology, and management of the western and northern corn rootworm complex has and is continuing to generate knowledge useful for pest management decision making. A knowledge based decision support system is presented for the management of western and northern corn rootworms. The system consists of simulation models of rootworm phenology, corn phenology, and an expert system to assist in pest identification, record keeping, and pest management decision making. Spring treatment decisions are determined by previous adult counts and estimates of over winter egg survival, enabling producers to determine the potential for damage at planting. Late summer treatment decisions are determined by adult counts and predictions of adult emergence. Ancillary components include a record keeping system for weather data, pesticide usage, and scouting reports. Working together, these components assist producers in properly parameterizing component models, entering current and past weather information, and performing what-if scenarios related to their production practices.

22-145

FORECASTING PROTECTION OF VINEYARDS FROM LOBESIA BOTRANA SCHIFF. (LEPIDOPTERA: TORTRICIDAE)

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The intensive use of pesticides for vineyard protection from the grapevine moth - *Lobesia botrana* have led to environment pollution, decrease of biodiversity, appearance of resistance population of noxious organisms. Applying in vineyard against grapevine moth phosphororganic pesticides promoted the reproduction of *Shyzo-tetranychus pruni* on the large scale, decrease of predatory mites and other beneficial organisms.

In the result of research the forecasting protection of vineyards from grapevine moth based on reliable prognosis of phenology and dynamic population of *Lobesia botrana* was worked out. This method has made possible to decrease pesticide press and conservation of the beneficial fauna.

22-147

THE UTILIZATION OF INSECT-PROOF NETS IN ISRAEL'S AGRICULTURE

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During the last years, we have been using insect-proof nets in different agricultural systems as a strategy of pest control. Good pest control has been obtained in vegetables (specifically in tomato), Herbs and flower greenhouses, and in fruit orchards (dates, figs, pomegranate apple, mango, peach and grapes). Results from several experiments in all these crops, and from commercial agroecosystem that already established the use of nets as a standard agronomical practice (e.g. tomato), show that the main complex of insect pests, and insect vectors, attacking these crops is significantly reduced, or eliminated. Moreover, our experience shows that insect-proof nets highly improved the quality of the crop by its direct action on the reduction of insect pests, and by reducing the negative effect of other environmental factors (such as extra sun radiation, highstorm, frost, etc.).

22-148

THE DENSITY OF GROUND DWELLING ARTHROPODS IN IPM AND CONVENTIONAL APPLE ORCHARDS IN HUNGARY

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Ground dwelling arthropod assemblages were studied in integrated /IPM/ and conventional /CON/ apple orchards by pitfall trapping in 1992 - 1994 in Hungary.

- The density of *Heteroptera* and *Coleoptera* /both predator and phytophagous/ assemblages were higher in IPM blocks.
- The densities of *Dermaptera* were higher in the IPM blocks in 1992 - 1993, on the other hand, they were more abundant in the CON block in 1994.
- The spider assemblages were not different in size /IPM-CON/ in 1992 - 1993, but in 1994 the number of individuals was higher in IPM blocks.
- The size of the *Orthoptera* assemblages were higher in IPM blocks only in the year of the outbreak, in 1993.

22-150

DAMAGES OF *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA-PLUTELLIDAE) ON BROCCOLI IN APULIA AND POSSIBILITY OF INTEGRATED PEST MANAGEMENT.

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Some years *Plutella xylostella* (L.) (Lepidoptera Plutellidae) proves to be one of the most harmful pests of broccoli (*Brassica oleracea botrytis* L.) in Apulia (South Italy). This is one of the autumn-winter vegetables among the most exported to Northern Europe where markets require pesticide residues-free products. Therefore it was necessary to evaluate the pest damages in the Mediterranean environment to find some parameters hampering its pullulations thereby reducing or eliminating the use of pesticides.

This work was carried out in 1994-95 in plantings located in Cerignola area (Foggia). Over these two years the following results were obtained : a) the actual damages caused by *P. xylostella* in nursery beds where a very high infestation percentage was observed (100%) during the peak population period; b) after transplanting, a reduction of damages induced by the lower infestation percentage on more developed plants; c) the impact of climatic factors showing that plants can escape the pest damages by postponing the growing season; d) the presence of some parasitoids.

22-149

EFFECT OF LEVEL OF DEFOLIATION ON RECOVERY OF LEAF AREA AND ON YIELD AND AGRONOMIC TRAITS OF SOYBEAN *Glycine max* (L.) Merrill

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Four levels of defoliation (0, 33, 67 and 100%) were applied at four stages of development (V_3 , V_8 , R_2 and R_6) of soybeans - cv. Paraná, at EMBRAPA/CNPSo experimental station (Londrina-PR, Brazil). Defoliation was made by hand, cutting one leaflet of each leaf for each 33% of defoliation. Recovery of leaf area was measured at five and 12 days after defoliation, and mathematical simulations were made to study parameters of recovery. Results indicated intense recovery measured by liquid daily rate of leaf area increase (dr) and recovery time (rt) for low defoliation levels, specially when applied at vegetative stages. During the reproductive period, levels of defoliation reduced the rate of the soybean natural trend of losing leaf area. Yield was affected only by 67 and 100% defoliation applied at R_6 , while main agronomic traits such as date of harvesting maturity, plant lodging and plant height were not affected by the treatments.

22-151

COMPARISON OF SOYBEAN INSECTS SAMPLING METHODS. II. PREDATORS, DIPTERA AND HYMENOPTERA.

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The shock technique is a variant of the beat cloth method of sampling soybean insects, developed to overcome the disadvantage of the original method, of heavily underestimating population of flying adults of several orders, specially Diptera and Hymenoptera. To compare the results of the two sampling methods, four experiments were set up during 1992/93 soybean season and one during 1994 season, while during 1995 both methods were compared to the sweep net. All experiments were set up in the Londrina region (Paraná-Brazil). Results indicated that beat cloth and shock methods are similar when sampling predatory arthropods, but the shock method proved to be more efficient when sampling species of Diptera and Hymenoptera. Based on these results, the shock method is recommended when sampling these insect orders, specially when involving biological control evaluations, or insecticide selectivity to predators and parasitoids.

Section 23

Medical and Veterinary Entomology

23-002

VECTOBAC 12AS, AN ENVIRONMENTALLY FRIENDLY MOSQUITO CONTROL SUBSTANCE

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In view of the importance of sheep farming in South Africa and the incidence of stock diseases related to increased mosquito numbers during years of high rainfall together with the fact that various mosquito species can act as vectors of various human diseases it is important to find an effective, environmentally friendly and economically affordable control method for mosquitoes.

In evaluating VectoBac 12AS, laboratory and field surveys were done using various concentrations of VectoBac 12AS. Laboratory reared larvae of *Aedes aegypti*, *Eretmapodites quinquevittatus*, *Culex theileri*, *Cx. quinquefasciatus*, *Culiseta longiareolata* and field collected *Anopheles squamosus*, *An. cinereus*, *An. coustani* and *Cx. theileri* were used in the laboratory trials. After treatment with 0,05 ml VectoBac 12AS/m², 100% mortality occurred within 12 hours. However, 1,3% of *Er. quinquevittatus* and 16,7% of *An. cinereus/coustani* survived for 5 days after exposure to 0,25 ml VectoBac 12AS/m².

Under field conditions *Cx. theileri*, *Cs. longiareolata* and *An. squamosus* showed up to 80% mortality within 24 hours after treatment with 0,5 ml VectoBac 12AS/10 m². After treating with 2,5 ml VectoBac 12AS/10 m² more than 85% of the larvae died. The pupal stage was not affected by VectoBac 12AS.

Natural occurring non-target organisms e.g. Ephemeroptera larvae, Odonata larvae (Anisoptera & Zygoptera), Hemiptera adults and larvae (Notonectidae & Geridae), Coleoptera larvae (Dytiscidae), Copepoda and Amphibia (*Xenopus laevis*) were exposed to VectoBac 12AS at doses up to 5 ml VectoBac 12AS/m². No treatment related mortalities occurred. However, chironomid larvae (Diptera) were affected by VectoBac 12AS. At a dosage of 0,5 ml VectoBac 12AS/10 m² a mortality of more than 90% occurred.

From the results obtained it is concluded that VectoBac 12AS is a very effective substance to control mosquito larvae. At a cost of approx. US \$5 to treat one hectare of water, VectoBac 12AS is also an economically affordable product for mosquito control.

23-001

ACYLATED 1,3-AMINOPROPANOLE DERIVATIVES AS REPELLENTS AGAINST BLOOD SUCKING ARTHROPODS - STRUCTURE/ACTIVITY-RELATIONSHIPS AND EFFICACY IN THE FIELD

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m-Diethyltoluamide (Deet), an effective repellent against blood-sucking Diptera, has been used widely throughout the world for the last 40 years.

However, this compound is irritating to mucosa and aggressive towards some plastic materials. Our aim was to find new compounds with at least the same efficacy as Deet but without these disadvantages.

The chemical synthesis of the title compounds, which split into several groups with high biological activity, and molecular modelling aspects are described.

The substances were screened for repellent activity against *Aedes aegypti* on guinea pigs, and those giving a longer protection period than Deet were further tested against *Anopheles stephensi*, *Culex quinquefasciatus* and *Stomoxys calcitrans*.

Structure/activity relationships on *Aedes aegypti* revealed hydroxypiperidines acylated at the nitrogen and with a special spatial relationship of the two oxygens as well as a lipophilic side chain attached to the carbonyl group to be the most effective compounds.

The best substance (1-Piperidinecarboxylic acid, 2-(2-hydroxyethyl)-, 1-methylpropyl-ester, lab code KBR 3023) in laboratory tests on guinea pigs proved superior to Deet against all insect species tested and also with respect to irritation of mucosa and neutrality towards plastics.

In field trials this compound was demonstrated to be equal or superior to Deet against many dipteran insects and ticks.

23-003

BEHAVIOUR OF THE HORN FLY AND ALTERNATIVE CONTROL STRATEGIES

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Traditionally the horn fly *Haematobia irritans* (L.) is controlled by using insecticides. However, due to e.g. insecticide resistance problems, concern for the environment, and the use of organic farming methods there is an increasing need for alternative methods for control of this and other cattle-visiting fly species.

For this reason the behaviour of the horn fly has been studied, in particular the individual variation between cattle in resistance to horn flies, constancy of fly distribution over time and dispersal of hornflies within a herd. Data was produced by 2 to 4 daily observations of hornflies on individual heifers in two herds of cattle. The observations were made during August in two consecutive years. Flies were counted in five different areas of the heifers' body - head, back, side, belly and legs. For the dispersal studies horn flies were caught from one of the most fly susceptible cattle, marked with a fluorescent colour and then released onto the same heifer again. The dispersal of the marked flies within the herd was then followed for up to 24 hours.

Based on produced data trials were made to estimate the potential of manipulating horn fly behaviour. In this experiment the most horn fly susceptible cattle in one herd were interchanged with the most horn fly resistant cattle in a second herd. The fly-load per cattle and per herd was studied before and after the replacement. It was shown by the investigation, that it is possible to manipulate the fly-load per herd by changing the composition of fly-susceptible and fly-resistant cattle within the herd.

23-004

THE USE OF CHEMICAL AND VISUAL ATTRACTANTS TO OPTIMISE THE EFFICIENCY OF A CONTROL SYSTEM FOR THE HOUSE FLY, *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE), IN POULTRY UNITS IN SOUTHERN ENGLAND.

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The house fly, *Musca domestica*, is an important pest in poultry units, where its presence in large numbers can constitute a considerable nuisance and public health problem. The traditional control technique used in intensive animal rearing units is the application of residual insecticides. Continued use of toxicants has resulted in high levels of resistance developing to all the major groups of contact insecticide employed. This problem has become particularly acute in southern England, where many poultry farms have become populated with multi-resistant strains of *M. domestica*. For these reasons attention has recently switched to control methods that minimise the use of insecticides.

One such method is to present the insecticide in localised toxic baits, where the mode of action is through ingestion of the toxin. This results in a reduced level of insecticide released into the environment, and lower selection pressure for resistance to appear. However, for toxic baits to form an efficient component of a control strategy they must attract *M. domestica* in sufficiently large numbers.

M. domestica sex pheromone, (Z)-9-tricosene, is known to have potential for increasing the attractiveness of toxic baits. Field trials have therefore been conducted to investigate the effects of providing visual cues, sex pheromone and food odours on the attractiveness of toxic bait traps. The results of these trials, and their implications for house fly control, will be discussed.

23-006

QUALITY OF FILTH FLY PARASITES RELATIVE TO EFFECTIVENESS

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Pteromalidae have been sold for filth fly control in the US with numerous user questions and complaints relative to their effectiveness. During 1982 we began monitoring the species, emergence % and numbers released in confined cattle facilities. The species was different than claimed, emergence was low and variable, and the number delivered was much less than needed. To correct these problems we sampled feedlots, determined the natural parasite complex present, then developed rearing methods to produce the dominant pteromalid species attacking the stable fly, *Stomoxys calcitrans* (L.).

Mass releases of *Spalangia nigroaenea* Curtis in an IPM system have been successful in reducing stable flies below economic damage levels. This species has been the primary parasite of stable flies, even when other species were being mass released. It appears to be better adapted for searching and finding stable fly pupae for oviposition. *Muscidifurax* spp. have been recovered from house fly, *Musca domestica* L., pupae (90% of the sampled *Muscidifurax*) with very low numbers from stable fly pupae. Release of *Muscidifurax* spp. has been less successful for house fly or stable fly reduction.

23-005

AUTOSTERILIZATION OF THE HOUSE FLY, *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE) IN POULTRY HOUSES IN NORTH EAST INDIA.

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Autosterilizing devices attract and sterilize wild flies in the field either directly, using chemosterilants, or indirectly, through transovarial disruption of the development of eggs, larvae or pupae. Some of the earliest autosterilizing systems developed for control of the cosmopolitan pest *Musca domestica* (Diptera: Muscidae) used alkylating agents such as tepa, metepa and apholate as chemosterilants. However, alkylating agents have never become widely used due to their high toxicity and minimal insect specificity, resulting in difficulties with safe deployment in the field.

More recently, a number of insect growth regulators (IGRs) have shown considerable potential as alternatives to the alkylating agents for use in autosterilizing systems. For the house fly, the chitin synthesis inhibitor, triflumuron has been shown to prevent egg hatch and larval development. Furthermore, exposure of female *M. domestica* to triflumuron on sucrose-baited targets in the laboratory induces significant transovarial ovidical and larvicidal effects and, following similar exposure, males are also capable of impairing the reproductive performance of unexposed females.

Field trials were therefore been carried out to evaluate the use of triflumuron treated, sugar-baited targets for autosterilization of *M. domestica* in poultry houses. The results of this fieldwork will be discussed in relation to the practical application of this technique for house fly control in livestock systems.

23-007

INTEGRATED PEST MANAGEMENT OF *DERMATOBIA HOMINIS* LIN. JR., 1781 (DIPTERA: CUTEREBRIDAE) IN LATIN AMERICA

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Dermatobia hominis is one of the most important ectoparasites attacking cattle in Latin America. Studies on biology, ecology and chemical control of this insect have been carried out in our university. Based on these data we can suggest the following elements to be integrated for controlling *Dermatobia*:

1. When substantial larvae populations are present in cattle, insecticides treatments are the only recourse. The most appropriate insecticides are organophosphates, ivermectins, closantel or fenil-pirazol.
2. Keep clean the pasture lands to avoid the presence of micro habitats formed by bushes and weeds, which protect *Dermatobia* adults and their vectors.
3. Install traps to capture dipteran vectors, which carry *Dermatobia* eggs.
4. Reduce larvae of dipteran vectors of *Dermatobia* eggs associated with feces, treating cattle with IGR boluses.
5. Chemical control of ticks using new pyrethroids must be recommended, because these substances show good repellent effect on dipteran vectors and kill first instar larvae of *Dermatobia*.
6. *Dermatobia* resistant cattle like light color Zebu, should be recommended for highly infested regions, because dark colour animals are more attractive to the vectors of *Dermatobia* eggs.

23-008

WHERE DOES *ANOPHELES ARABIENSIS* BITE?

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Anopheles arabiensis Patton is the most important vector of malaria in southern Africa, yet little is known of its biting behaviour. A seasonally-abundant population of these mosquitoes at an isolated freshwater spring in north-eastern South Africa has allowed unique study opportunities.

More than 90% of *An. arabiensis* bites occur within 15cm off the ground. This means that in normal standing or seated position most bites occur on the feet and ankles. Our studies have also shown that covering the feet and ankles greatly reduces the number of bites. If prevented from feeding on ankles and feet, equivalent numbers of *An. arabiensis* do not move up to bite higher on the legs, but rather seek feeding opportunities elsewhere.

Exposure to *An. arabiensis* can be significantly reduced by covering feet and ankles, and by constructing residential units on stilts in areas having high risk of malarial infection by these mosquitoes.

23-010

FLYING UPWIND TOWARD ODOR PLUMES

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Flying upwind to the source of an odor presents the challenge of continued detection of the wind's direction. An upwind course is set by optomotor anemotaxis, that is using a visual appraisal of how wind modifies the insect's path. A front-to-rear flow directly below an organism occurs when progress is due upwind. Details of this process have emerged mainly from wind tunnels studies of male moth flight to pheromone. In the field, changes in wind direction often cause a moth to lose contact with the scent, triggering casting, or a series of widening zigzags without upwind displacement. Continued contact with the odor plume yields upwind progress, usually in the form of zigzagging, a temporally regular pattern of counterturns across the windline. In moths, the precise course appears governed by moment-to-moment contact with odor. A brief contact with a pheromone filament causes a surge toward upwind, and an optimal rate of encountering filaments yields a course nearly straight upwind. Other insects such as parasitoid wasps 'seeking' a host do not seem to have such counterturning programs and often fly straight upwind. The success of these maneuvers in odor location is influenced by the pattern of odour dispersion, wind variability, and the availability of visual cues.

23-009

WIDESPREAD DISTRIBUTION OF INSECTICIDE IMPREGNATED CURTAINS ENTAILS A DRAMATIC REDUCTION OF MALARIA TRANSMISSION IN A HYPERENDEMIC AREA OF BURKINA FASO*

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In a large scale trial involving 158 villages (c. 100000 population) in a rural area of Burkina Faso, insecticide impregnated curtains were installed in randomly selected clusters of villages. Each cluster included about 10 villages and covered an area of about 55 km². In two sentinel villages located in clusters receiving the intervention, the number of *Plasmodium falciparum*-infected *Anopheles* bites during the period from August to December was reduced from 444 to 32 and from 1252 to 36 following the intervention. After curtain installation, the circulating *Anopheles* populations decreased by more than 50% and sporozoite rates fell from 9.1% to 2.7% and from 13.2% to 5.7%. Similar falls were not observed in 2 control villages. We estimate that the individual protection against biting afforded by the curtains was of the order of 50% and 90%, and that the impact of the control measure on the vectorial capacity resulted in an overall protection against infected bites of 93% and 97%. These results indicate that, at least in areas of high transmission, a high level of coverage over a wide area may provide substantially greater protection against malaria transmission than the individual protection can offer alone.

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23-011

THE EFFECTS OF PLUME FINE-SCALE STRUCTURE UPON PHEROMONE-MEDIATED FLIGHT BEHAVIORS OF MALE MOTHS

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Manipulations of the gross structure of pheromone plumes have long been used as an approach to understanding the behavioral mechanisms used by male moths to locate a calling female. Recently, experiments varying features of the temporal fine-scale structure of pheromone plumes have proved to be valuable in further dissecting those behavioral mechanisms. Two, independent studies have shown, in different species, that male moths are capable of responding to small wisps of pheromone (known as filaments) by making an upwind surge followed by a return to crosswind, casting flight. In multiple pulse plumes, generated at a threshold frequency, upwind progression by the Noctuid moth *Heliothis virescens* (F.) appears to be constituted by many such upwind surges with casting appearing as a function of the clean air gaps between filaments and the male's behavioral latency. Furthermore, in this same species of moth, filaments tainted by the presence of a novel inhibitor produced surges that are diminished compared to those occurring in response to normal blend filaments, providing a possible explanation for the reduced levels of upwind flight and source location that occurred in similarly tainted point-source plumes, where males would contact many such contaminated filaments per second.

Stationary electroantennogram (EAG) preparations have been used to determine how plume fine-scale structure can vary in differing physical conditions. In addition, we investigated the magnitude and frequency of pheromone filaments that a male moth might encounter during upwind flight in a plume by using an EAG attached to a flying male. Simultaneous video-recording of the olfactory response of the transported antenna and the male's flight path facilitated a correlation between olfactory input and behavioral output.

Our behavioral experiments underscore that the upwind flight performance of a male moth is strongly influenced by changes in both the temporal structure of a plume and the quality of moment-to-moment olfactory contacts.

Current neurophysiological investigations are aimed at understanding how central olfactory pathways in male *H. virescens* respond to changes in the temporal structure and olfactory quality of pheromone plumes.

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23-012

DISPERSION OF HOST ODOUR PLUMES: MICROMETEOROLOGICAL STUDIES IN NATURAL HABITATS OF TSETSE FLY

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Tsetse fly in its natural habitat (African bush) uses carbon dioxide and some other gaseous constituents in plumes of host odour as chemical stimulus to find its host. There is laboratory evidence, that tsetse may not only react to step-wise increase of CO₂ concentration (over CO₂ background), but also to the (turbulent) fluctuations in the received 'CO₂-signal'. Besides the chemical stimulus, the odour plume provides also directional stimulus due its three-dimensional (turbulent) structure. Since surface layer turbulence determines (in-canopy) dispersion, it should also influence host finding of tsetse. At Rekomitjie Research Station (Zimbabwe) the dispersion and structure of CO₂-plumes was investigated by sensitive and fast response (<0.1s) measurements of CO₂ concentration and extended micrometeorological measurements (turbulent fluxes, vertical profiles of air temperature, wind speed and -direction) at several positions downwind a CO₂-source. During daytime the background noise of CO₂ was surprisingly low (< ±0.5 ppmv/5 min), which generally favours the detection of host CO₂ at considerable distances from the host. Detectable CO₂-signals were found even at >60m from the CO₂-source. Micrometeorological measurements of vertical temperature gradients have demonstrated the strong influence of surface layer stability on plume dispersion within the canopy. During unstable conditions (late morning to early afternoon) any released material was dispersed rapidly by thermal buoyancy forces to background concentration levels. However, the distant detection of host odour was mostly favoured under stable and neutral conditions (early morning and late afternoon), when the same material was kept very close to the surface during its turbulent transport through the bush.

23-014

THE SPEED AND EFFICIENCY OF ODOUR SOURCE LOCATION BY TSETSE FLIES (*GLOSSINA PALLIDIPES*); A MARK-RELEASE VIDEO STUDYN. Griffiths, J. Brady, Q. Paynter²Dept of Biology, Imperial College, Silwood Park, Ascot, Berkshire, UK
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Individually marked tsetse flies (*Glossina pallidipes*) were released under video observation, in medium density bush, from 30-75 m downwind of an odour source and recaptured at the source by an electric net. In the absence of odour the proportion recaptured was <2% (approximately equal to random expectation from radial dispersal). When synthetic ox odour was emitted the proportion recaptured increased with proximity of release from 6% at 75m to 21% at 30m (though supplementary work implied around twice this number of flies entered the clearing by the odour source). The probability of recapture decreased with increasing wind speed but increased with the directness of the wind prior to take-off.

A clear distinction could be made between the release-recapture times of flies recaptured with odour. A 'fast' cohort of flies (approximately 50 % of recaptures) arrived within 40 s, with times normally distributed around a mean which increased linearly with release distance giving a mean rate of (straight line) upwind displacement of 2.8 ms⁻¹. The other 50% of recaptured flies were 'slow' - between 1 and >20 min. with a constant probability of arrival through time. No wind or temperature correlates with in-flight time were found although the probability that a fly was 'fast', decreased with increasing 'in-flight' wind speed.

The random nature of the 'slow' arrivals through time, presumably having lost the plume at some stage and/or rested on the way prevents any further inference of how they located the source. However the displacement rates of 'fast' flies together with the low probability of recapture with no odour imply they must have reached the odour source in a single uninterrupted flight step contacting the odour at some point from take-off to recapture. We infer that a form of biased random walk has been used to bring the insect within sight of the source, incorporating a combination of anemotactic 'aim-then-shoot' orientation from take-off plus optomotor-steered in-flight correction of direction.

23-013

EFFECTS OF WIND SPEED ON THE OLFACTORY FINDING OF HOSTS BY TSETSE FLIES (*GLOSSINA*)

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Wind speed in thick vegetation is slow (0-1 m/s). In such situations, it is highly non-linear (typically changing direction by > 10 °/s). As it increases speed (up to 1 m/s), however, its flow straightens out. When the wind in vegetation increases, therefore, odour plumes should be straighter, and hence be easier for insects to follow upwind to the source of the odour (whether mate or host).

This suggests that host-seeking insects such as tsetse flies should find it easier to locate hosts by odour in the faster winds of their bush habitat (i.e. in winds approaching 1 m/s (= ± straight)) than in the more typical weaker winds of their habitat (of around 0.3 m/s (= meandering)). This prediction was tested in the field in Africa several times, by means based on correlating the rate of arrival of tsetse flies at an odour source with the concurrent wind speed measured in various ways.

The real situation was more complicated: the relationship between source finding success and wind speed appeared to be bell-shaped. In some situations, arrival rate and wind speed correlated significantly positively (supporting the prediction), though always with low coefficients ($r^2 < 20\%$). In other situations, the same correlations were significantly negative (refuting the prediction).

Overall, the conclusion drawn is that - for tsetse flies at least - as the wind increases from near zero towards ca 0.5 m/s, flies do indeed find an odour source more easily. But as the wind increases to speeds above ca 0.7 m/s, the flies find it harder to locate odour sources, presumably because of the odour plume's greater dilution and fragmentation in the faster, more turbulent winds.

23-015

THE DYNAMICS OF AN ISLAND POPULATION OF TSETSE FLIES (*GLOSSINA* SPP.) IN ZIMBABWE

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A mark-recapture exercise, carried out on Antelope Island between October 1979 and December 1983, provides a unique series of more than 200 consecutive weekly estimates of births and deaths, marked and total populations, and probabilities of capture and recapture for male and female *G. m. morsitans* Westwood and *G. pallidipes* Austen. Two approaches have been taken to the further analysis of the data. First, multiple regression has been used to produce a function which predicts the survival probabilities of adult and immature tsetse in terms of the known conditions on the island. (Maximum temperature and trapping intensity were shown to be the major determinants). Secondly, a new approach to the modelling of population data has been developed. The entire history of growth and decline was modelled using a stochastic process, starting from the pupae originally introduced onto the island. The life of each pupa was simulated using a Monte Carlo procedure. For both sexes the procedure made a daily test of survival; for females it also tested for the production of new pupae - whose life histories were, in turn, simulated. By including the birth process it was possible to model the male and female populations simultaneously. The survival function referred to above was used as a starting value for the procedure. The innovation comes in the linking of this procedure with a non-linear programme, whereby the parameters of the survival function can be optimised iteratively via a least squares fit to the whole series of (male and female) population estimates. By including the capture probability in the simulation process it is possible to model changes in the marked population at the same time.

23-016

ORIENTATION OF BLACK FLIES (DIPTERA: SIMULIIDAE) TO HOST ODOURS

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Like most other haematophagous insects, black flies combine various host odours with visual and other cues to locate and select hosts. Important host odours for some black flies include carbon dioxide, non-CO₂ breath odours, and body odours. This paper reviews the last fifty years of field research into the nature and role of host odours in black fly host seeking. The following questions are addressed: 1) What roles do host odours have in host location by black flies in different habitats? 2) Are host odours important in determining preferred host species and individual? 3) Are odour cues important in determining the level of black fly attack on different hosts? 4) What mechanisms do black flies use to follow odour plumes?

23-018

Olfactory hostfinding of yellow fever mosquitoes *Aedes aegypti* (Diptera: Culicidae): Synergetic effect of different host odour components.

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In a Y-tube wind tunnel, mosquitoes fly upwind towards host odour sources such as a human hand and also towards an extract obtained by rubbing the hands and feet with a wad soaked in ethanol. If we offer these stimuli in one of the two branches of the Y-tube, we are able to trap 80 to 100 % of the mosquitoes on the test side within 30 sec after stimulus onset. In the most effective dose, the extract is about as attractive to mosquitoes as a human hand. We used this behavioural response as a bioassay to evaluate the attractiveness of liquid-chromatography-fractions of this extract. Lactic acid was confirmed to be a major constituent. Our results demonstrate, that lactic acid is a necessary component for the extract's effectiveness. After its removal, the extract is no longer attractive. However, without the other odour components lactic acid is only slightly effective. This indicates that the extract's high degree of effectiveness is based on a synergism of lactic acid and other odour components. Liquid chromatography of the extract revealed three distinct regions of active fractions. One region contained lactic acid but the effective odours of the other two regions are not identified as yet. A combination of these fractions together with lactic acid is as attractive as the complete extract. These results clearly support an olfactory host recognition using a complex stimulus pattern.

23-017

THE INTERACTION BETWEEN HOST ODOURS AND CARBON DIOXIDE IN HOST SELECTION BY WEST AFRICAN MOSQUITOES

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Carbon dioxide is a potent mosquito kairomone, both by itself and, in some cases, as a synergist with other odours -- e.g. L-lactic acid, 1-octen-3-ol. Indeed, no molecules of a potency approaching that of carbon dioxide when used alone, have been discovered so far. The role of other host odours relative to that of CO₂, however, is likely to vary according to the feeding habits of each mosquito species. Strictly anthropophilic malaria vectors as *Anopheles gambiae* s.s., whose odour-mediated host preference for humans has been confirmed, respond to man-derived air-borne cues other than CO₂ more 'effectively' as compared to apparently opportunistic feeders like *Mansonia uniformis*. Nevertheless, CO₂ appears to play an important role for both species in determining how many mosquitoes will eventually approach the odour source. In fact both species show similar dose-response curves for CO₂ in the field; moreover, CO₂ contributes to a large amount of the difference in attractiveness between single human odour-baits. Given the recognised role of CO₂ in the activation and, possibly, upwind displacement of mosquitoes, differences in the amount of carbon dioxide released by a host are likely to have active space/recruitment effects. These are in turn modulated by host odours according to the host selectivity of each mosquito species. Endophagic, nocturnal mosquitoes like *An. gambiae* s.s. and *An. arabiensis* are confronted with a new environment once they have entered the hut where the host(s) sleep(s). Wind is no longer available and visual cues are probably scarce for optomotor anemotaxis; moreover, new short-range physical/chemical cues become accessible. Our experiments in the field have so far dealt only with the outdoor long-range behaviour of mosquitoes, and the results should thus not be extrapolated to include the indoor situation.

23-019

THE EFFECT OF PHYSICAL FACTORS ON HOST-LOCATION BEHAVIOUR OF MOSQUITOES (DIPTERA: CULICIDAE)

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Host-location behaviour of mosquitoes is influenced by physical, visual and olfactory stimuli. Whereas knowledge on the effects of visual and olfactory stimuli is advancing rapidly, the influence of physical factors is poorly understood. This paper describes a laboratory study on the importance of temperature and humidity in host-location behaviour of the malaria mosquito *Anopheles gambiae sensu stricto* Giles. Five day old female *An. gambiae* s.s. were released in batches of 50 at a time in a large two-choice olfactometer. On the upwind side they were collected in glass-container traps, through which pre-conditioned air containing variable stimuli flowed into the observation arena. The response to variations in relative humidity were studied in the presence and absence of odour stimuli. There was no response with relative humidities below 40%. There was a significant response with increasing relative humidity compared to steady state or falling RH's. There was no response to 4.5% CO₂. Mosquitoes responded in large numbers to the emanations of a human hand (hand held in the air stream), but significantly more when RH was increasing than when it was falling or stable. It is concluded that *An. gambiae* s.s. uses a combination of physical and chemical cues in host location, and that slight elevations of moisture above background are required for optimal responses to olfactory cues. The relevance of these findings for field research will be discussed.

23-020

A MALE ACCESSORY GLAND PROTEIN MODULATES FEMALE MOSQUITO (DIPTERA: CULICIDAE) HOST-SEEKING BEHAVIOR

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The host-seeking behavior of gravid *Aedes aegypti* mosquitoes is modulated by mating and specifically by proteins transferred from the male accessory gland. Using gel filtration, we isolated and identified the component from male accessory glands responsible for this effect of mating. It appeared to be a protein between 5 and 10KDa, which when injected had the same effects on inhibiting host-seeking behavior as did mating.

23-021

PROGRESS IN THE DEVELOPMENT OF A RADAR FOR TRACKING PLUME-FOLLOWING INSECTS.

J.R. Riley, A.D. Smith

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Conventional radar is unsuited to tracking insects in low-altitude, plume-following flight, because radar reflections from the ground and from vegetation (clutter) completely obscure the tiny radar echoes from insects. We have therefore attempted to overcome this problem by using the harmonic radar principle. In this technique, the target to be tracked carries an electronic tag which picks up energy from incoming radar pulses, and retransmits some of this energy at twice the radar frequency. This frequency-doubled signal can be detected even in the presence of vastly stronger clutter echoes, and so low-flying targets can be tracked. The technique has the great advantage that no on-board battery is needed, and so extreme miniaturisation of the tag is possible. Initial tracking experiments with electronically tagged bees have been promising¹, and in this presentation we describe progress towards producing a tag for use on smaller insects, such as tsetse flies.

References:

1. Riley, J.R., Smith, A.D., Reynolds, D.R., Edwards, A.S., Osborne, J.L., Williams, I.H., Carreck, N.L. & Poppy, G.M. (1996) Tracking bees with harmonic radar. *Nature* 379, 29-30.

23-022

Symposium: Mosquito Producing Wetlands: A United States Problem with Global Implications.

Organizers: W.A. Rowley, R.J. Novak, and J.K. Olson

Moderator: R.J. Novak

ABSTRACT:

Conservation of natural wetland habitats is required to sustain the vitality of the environment as well as to preserve the diversity of animal and plant species supported by these particular kinds of habitats. One hundred and fifty years ago, wetlands were abundant throughout the United States and the world, especially along the major river-drainage systems. However, expansion of agricultural and urban land uses, particularly over the last century, has dramatically reduced the number, diversity, and size of these habitats. The establishment, re-establishment, and maintenance of extensive wetland habitats in proximity to urban and rural communities in ways that will facilitate multi-purpose uses requires the development and implementation of economical sound and environmentally safe mosquito management strategies. These strategies must take into account and ensure not only the protection of human health and quality of life, but also provide for the protection of natural and domestic animals and plants.

The objectives of this Mosquito-Wetlands Symposium are to: 1) present bionomic information on major wetland mosquito species, 2) compare and contrast natural and man-made mosquito producing wetlands ecotypes, 3) discuss mosquito control and associated environmental problems, and 4) present the human and animal health risks associated with mosquito-borne pathogens.

23-023

AEDES VEXANS: A NUISANCE AND VECTOR WETLAND SPECIES

R. Novak (Champaign-United States)

23-024

AEDES TRIVITTATUS: A NORTH AMERICAN WOODLAND-WETLAND SPECIES

W. Rowley (Ames-United States)

23-025

EPIDEMIOLOGY OF ARBOVIRUSES ASSOCIATED WITH WETLAND MOSQUITOES

K. Platt (Ames-United States)

23-026

WETLAND ANOPHELES: THE ANOPHELES QUADRIMACULATUS COMPLEX

L. Meek

23-027

WETLANDS CONSERVATION AND ITS IMPACT OF MOSQUITO CONTROL

R. Washino (Davis-United States)

ABSTRACTS NOT RECEIVED

23-028

WETLANDS AND THEIR IMPACT UPON VECTOR-BORNE DISEASES

A.B. Knudsen¹, Symposium Mosquito producing wetlands, Robert J. Novak, Organizer

¹Scientist/Medical Entomologist, World Health Organization, Division of Control of Tropical Diseases, Geneva, Switzerland

Historically, wetlands or marshlands have been viewed as areas associated with significant health risks due to an abundance of habitat most propitious for producing mosquito and other disease vectors, resulting in the transmission of malaria, lymphatic filariasis, leishmaniasis, arboviruses, etc.

Today, in some areas of the globe, where there has been favorable bio-rational policies in wetlands regulating man-made environmental changes, wetlands have been improved through appropriate management including effective drainage systems which enable periodic flushing of marshlands, which impacts upon the production of insects of public health concern. Such policies, when correctly applied and used in harmony with the selective use of biocides as an auxiliary control measure is in harmony with wildlife management principles. The results is a bio-healthy wetlands with a reduction in mosquito densities and a natural decline in vector-borne disease transmission.

However, where wetlands or marshlands continued to be administered in the traditional manner in the absence of well planned management guidelines, where flooding occurs periodically accompanied by an increase in pollution and obstruction of drainage, vector breeding is continues and in the absence of a consistent mosquito prevention and control policy, there is an associated increase in disease transmission. This presentation reviews the significance of wetlands, the need to conserve such natural resources while judiciously controlling vectors of disease.

23-030

MOSQUITO CONTROL LEADING INTO THE 21ST CENTURY

V. Meisch

ABSTRACT NOT RECEIVED

23-029

CONSTRUCTED WETLANDS: THE MOSQUITO PEST AND DISEASE THREATS, AND CONTROL OPTIONS

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Increasingly, wetlands are being constructed and used as a process to 'polish' urban storm and waste waters before their disposal into natural water systems.

These 'artificial' wetlands are also seen as an attractive urban asset, providing wildlife refuges and passive recreation areas.

However, mosquitoes exploit such habitats as they do more 'natural' wetlands, and thus can a significant nuisance and potential health risk to nearby residents.

The design of a wetland is critical to its potential for mosquito production; water and vegetation management can be effective for reducing mosquitoes but structural measures for mosquito control often appear incompatible with the objectives and operations of constructed wetlands.

The health issues and disease risks are not inconsequential, however, and should not be dismissed; mosquito surveillance and control should be important objectives in planning, construction and maintenance of wetlands.

This paper will address the issues of mosquitoes and constructed wetlands, with reference to various problems and some case studies in different countries.

23-031

LYME BORRELIOSIS IN EASTERN USA

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Human Lyme disease in eastern North America is caused by the spirochete *Borrelia burgdorferi* sensu stricto. It is transmitted from animals to humans in that region principally by the tick *Ixodes scapularis*. The tick is known to feed on a large variety of mammals, birds, and reptiles. Larvae and nymphs feed on small, medium, and large mammals, birds, and lizards; adults feed on large and medium sized mammals. *Borrelia burgdorferi* has been isolated from several small and medium sized mammal and bird species, however, the mouse *Peromyscus leucopus* is the most important reservoir in the northeastern and north central United States. Other *Peromyscus* species such as *P. maniculatus* and *P. gossypinus* are also excellent reservoirs in areas where they occur. Until recently, Lyme borreliosis was presumed to be absent or rare in the southern USA. The presumption was incorrect. *B. burgdorferi* is widely distributed throughout the south and several enzootic cycles involving several reservoir hosts and tick species are present. Much greater genetic diversity exists among southern spirochetes than among northern strains and there might be several genospecies present. Moreover, a greater diversity of tick species have been found infected. *B. burgdorferi* has been isolated from *I. scapularis*, *I. affinis*, *I. minor*, *I. dentatus*, and *Amblyomma americanum*. Antibodies to *B. burgdorferi* found among several species of animals further confirm the wide distribution of the spirochete in the southern USA.

23-032

BORRELIACIDAL FACTOR IN THE SERUM OF THE WESTERN FENCE LIZARD (*SCELOPORUS OCCIDENTALIS* BAIRD & GIRARD)

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The western fence lizard, *Sceloporus occidentalis* Baird & Girard, is a significant host of subadult *Ixodes pacificus* Cooley & Kohls, the primary vector of the Lyme disease spirochete in the far-western USA. Recent studies have shown that the prevalences of spirochetal infection in host-seeking *I. pacificus* nymphs sometimes exceed those in adult ticks. Experiments were conducted to determine if the reduced spirochetal prevalence in adult ticks is due to a borreliacidal factor in the blood of *S. occidentalis*. Ten *I. pacificus* nymphs having a high prevalence of spirochetal infection (~80%) were put on each of 10 lizards. Five of the lizards had been immunized previously with 10^8 heat-killed spirochetes, the other five lizards served as controls. Following repletion and the transstadial molt, none of the resultant adult ticks from all 10 lizards was found to contain spirochetes. In contrast, infected nymphal ticks that had fed on rabbits passed spirochetes transstadially. In a second trial, motile spirochetes were placed in sera obtained from lizards and several mammalian species. Most spirochetes put in lizard sera died within one hour, whereas those put in mammalian sera survived for up to 72 hours. If spirochetes were put in lizard sera that had been preheated (100 C for 10 min) first and allowed to cool, then they survived for up to 72 hours. I conclude that serum from western fence lizards contains a thermolabile borreliacidal factor. The presence of this factor, and not anti-spirochetal antibodies, appears to be responsible for reducing or eliminating spirochetal infections in *I. pacificus* nymphs that feed to repletion on *S. occidentalis*.

23-034

LYME BORRELIOSIS IN SOUTHERN EUROPE

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Ixodes ricinus is the main vector of *Borrelia burgdorferi* in Southern Europe. However, *I. hexagonus* and *I. canisuga* are also involved in enzootic cycles. *Apodemus sylvaticus* is probably the main reservoir of *B. burgdorferi* in Portugal. In one area three strains of *B. burgdorferi* have been isolated from *I. ricinus* and one of them is a new European genomic group (POTi B2). In Portugal no *B. burgdorferi* has been isolated from humans. In some regions from 5% to 12.5% of human population have antibodies against *B. burgdorferi*. The prevalence of cases seems to be around 5% of the human population. On Madeira island *I. ricinus* is infected with *B. burgdorferi sensu stricto*.

In northern Spain isolation of *B. burgdorferi* was made from *I. ricinus* and from a patient with erythema migrans. These strains are *B. burgdorferi sensu lato*. In southern Spain *I. ricinus* parasitizes 30% of wild carnivores and 4% of domestic bovines. Prevalence of spirochetes was from 11% to 28% in *I. ricinus* in northeast Spain. The human population had an antibody prevalence of less than 1% to 38%.

In Italy, *B. burgdorferi* antibodies were detected in 19.5% of asymptomatic rangers and forestry workers. *B. burgdorferi* was isolated from the blood of a patient suffering from a chronic form of polyarthritis in Italy.

In Slovenia 245 cases of Lyme borreliosis were registered in 1986.

The situation in Greece is not well known. However, three humans from Crete had antibodies to *B. burgdorferi*.

23-033

LYME BORRELIOSIS IN WESTERN, CENTRAL AND NORTHERN EUROPE.

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Lyme borreliosis (LB) is considered to be the most prevalent tick-borne disease in temperate areas of the Northern hemisphere. In Europe, unlike the United States, LB is not a notifiable disease and therefore our knowledge of the epidemiology of LB in this area is limited. The distribution of LB is correlated with the distribution of its main vector *Ixodes ricinus*.

European LB can be caused by three different genomic species: *Borrelia burgdorferi* (Bb) sensu stricto, *B. garinii* and *B. afzelii*. The division in genomic groups seems to have clinical relevance for LB in Europe.

Characterization of tick isolates of Bb suggests that Bb sensu lato contains more than three genomic species. Studies have demonstrated that various genospecies are present in the tick population of one endemic area and also that simultaneous infection by more than one genospecies can be detected in *I. ricinus* ticks.

Small rodents, birds, squirrels, hares and hedgehogs have been reported as reservoir hosts for Bb s.l. in Europe.

Bb has been detected in other tick species than *I. ricinus* but the role of most of them in the ecology of the disease remains unclear. However, *I. hexagonus* and *I. uriae* are able to maintain enzootic transmission cycles involving hedgehogs and sea-birds. The potential public health significance of the presence of Bb in these tick species, in areas where the bridge vector *I. ricinus* is absent, remains unclear.

23-035

LYME BORRELIOSIS IN THE FORMER SOVIET UNION Yu.S. Balashov

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In the area former Soviet Union Lyme borreliosis is spread throughout the entire forest zone from the western boundary up to the Pacific coast. The northern and southern boundaries of borreliosis are determined by geographic distribution ranges of vector ticks. The major vectors of borrelia to man are adults *Ixodes ricinus* and *I. persulcatus*. Most aggressive in attacking man is *I. persulcatus*. The season of borrelia transmission is determined by the period of activity of adults and coincides with May-July; in the areas of *I. ricinus* distribution it continues in August-September. Nymphs attack man much less frequently than adults. In the enzootic cycle of the pathogen participation of other close species of the genus *Ixodes* is possible.

Preimaginal phases of the first two species parasitize on several dozens of species of small mammals and birds and adults parasitize on average and large mammals. Borrelia were found on ticks using methods of dark field and phase contrast in many regions. Infestation of ticks varied from low percentage up to 80-90% and higher. Among mammal hosts borrelia were obtained from a few species of rodents and shrews. Borrelia were identified to a species in rare cases. *Borrelia burgdorferi sensu strictum*, *B. afzelii*, and *B. garinii* were found in ticks. These pathogens were found in both species of vectors and frequently in the same locality. Morphological techniques permitted to establish a great polymorphism in shape and sizes of borrelia in ticks. Localization, number and shape of borrelia in many cases depends on the stage of tick development.

23-036

LYME BORRELIOSIS IN JAPAN

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Since the first case of Lyme borreliosis (LB) was reported in Japan (1987), the total estimated number is about 100 cases until present time.

Almost all of those cases showed milder symptoms than the cases in the United States and /or Europe.

The cases appeared between April and August, reaching a maximum in June at Hokkaido. The LB of the patients was caused by infestation of adult female *Ixodes persulcatus* infected with *Borrelia burgdorferi* sensu lato.

In the spring, many people harvest edible plants in the field, and harvesters bring back the plants to their home together with infected ticks.

During an enzootic cycle the spirochetes are transmitted from the reservoirs (*Apodemus* mice, *Clethrionomys* vole and *Turdus*, *Emberiza* migratory birds) to another normal host by immature stage of *I. persulcatus* tick as an important vector.

No transovarial transmission of the agent of LB was observed.

The agents had been isolated from the erythema caused by tick bite in 19 LB patients removed infested ticks from their skin by themselves by using fingers before therapy.

23-038

SALIVARY GLAND ANTICOAGULANTS IN VECTOR MOSQUITOES (DIPTERA: CULICIDAE)

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Virtually all hematophagous arthropods salivate powerful anticoagulants, vasodilators, and platelet anti-aggregating factors that facilitate hematophagy. Anticoagulants in a number of important vector mosquitoes were demonstrated by a delay of coagulation of human plasma in the presence of female adult salivary gland extracts. Salivary gland extracts from all mosquitoes species examined caused a significant delay of the recalcification time, the prothrombin time, and the activated partial-thromboplastin time, indicating that they all exert their effects on the common coagulation pathway. Chromogenic assays to determine the coagulation inhibition site were performed for coagulation factor Xa (FXa) and thrombin, the major components of the common pathway. In a remarkable finding, all anopheline mosquitoes have thrombin-directed anticoagulants and the culicine mosquitoes have FXa-directed anticoagulants although variation exists in the degree of inhibition. For example, in the Yellow Fever mosquito, *Aedes aegypti* (L), the anticoagulant has been shown to result from the specific inhibition of FXa based on a FXa clotting assay and the inhibition of FXa-directed cleavage of the synthetic chromogenic substrate, chromozym X. The inhibition of FXa by female salivary gland extracts exhibits non-covalent, noncompetitive inhibition kinetics, and is reversible. We propose that the *Ae. aegypti* anticoagulant is a novel, proteinaceous serine protease inhibitor specific for FXa. The differences in the site of action of the anticoagulants most likely reflects the long period of independent adaptation of the two hematophagous mosquito subfamilies to the challenges presented by vertebrate hemostasis.

23-037

LYME BORRELIOSIS - A GLOBAL PERSPECTIVE: DISCUSSION, SUMMARY AND CONCLUSIONS.

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During this symposium on Lyme borreliosis, the presenting speakers will focus on the following topics: enzootic cycles; bridge (link) vectors between natural reservoirs and humans; genetic heterogeneity of *Borrelia burgdorferi* sensu lato; and the geographic distribution of the vectors (*Ixodes* spp.). Some of the main areas of frontline research related to the ecology and epidemiology of Lyme borreliosis during the next few years will be to investigate and explain:

. The geographic distribution of the different genomic species in the *B. burgdorferi* s.l. taxon, and the possible enzootic occurrence of this taxon and/or closely related taxa causing human "Lyme disease" in the Neotropical, Ethiopian and/or Australian region(s).

. The role of birds in the maintenance and dissemination of *B. burgdorferi* s.l.

. Factors determining the association between certain genomic species of *B. burgdorferi* s.l. and particular vertebrate reservoir species.

. The intrinsic biochemical and physiological factors determining competence or incompetence of potential vectors to transmit *B. burgdorferi* s.l.

. The development of mathematical transmission models to explain the dynamics of Lyme borreliosis in different geographic (climatic) regions, and mathematical models capable to predict human Lyme disease risk. In this context much research is needed to comprehend how various environmental parameters determine the geographically variable seasonal dynamics of the *Ixodes* vector populations.

23-039

VARIATION IN THE VASODILATORY ACTIVITY OF SALIVA AMONG POPULATIONS OF THE SAND FLY, *LUTZOMYIA LONGIPALPIS*.G.C. Lanzaro¹, A. Warburg², J-P Mutebi¹ and J.M.C. Ribeiro³

Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, TX USA-1, Department of Parasitology, Hadassah Medical School, Hebrew University, Jerusalem, Israel-2, Department of Entomology, University of Arizona, Tucson, AZ USA-3

The sand fly, *Lutzomyia longipalpis* is the vector of *Leishmania donovani chagasi* in Latin America. Working with three colonies, from Brazil, Colombia and Costa Rica, we conducted a series of studies which demonstrated that these three populations represent distinct species and that these species differ in the erythema producing quality of their saliva, with the bite of the Costa Rican species producing little or no erythema. We showed that this difference involves the vasodilatory peptide, maxadilan. We successfully cloned the maxadilan gene from each of the three putative species. An analysis of the nucleotide sequence from each species showed consistent differences between species and polymorphisms within species. Recombinant maxadilan was produced for each species and recombinant peptides were assayed for vasodilatory activity. All were found to have equivalent vasodilatory activity. These results suggest that the difference between Costa Rican flies and the others is not in the vasoactivity of the maxadilan peptide, but rather in the levels of expression of this gene. The occurrence of a fly species with limited vasodilatory activity in its saliva in Central America (Costa Rica) is coincident with a non-visceralizing disease produced by *L.d. chagasi*, which normally produces visceral leishmaniasis. We hypothesize that the development of visceral disease is influenced by the presence or absence of vasodilator in the vector saliva.

23-040

ANALYSIS OF BLACK FLY SALIVA AND ITS' RELATIONSHIP TO VECTOR STATUS

M. S. Cupp, E. W. Cupp

The vector capacity of medically-important *Simulium* species (black flies) is directly related to its' ability and propensity to blood-feed on humans. Because of the critical role of blood-feeding to vector status, we are analyzing and comparing pharmacological properties of black fly saliva from several species to identify activities that may be critical for vector competence.

Salivary glands extracts (SGEs) were prepared by salivary gland homogenization and centrifugation. The SGEs of all species examined contain potent vasodilative activity that was demonstrated by skin hyperemia following intradermal injection in the shaved skin of New Zealand White (NZW) rabbits. Total gland activity of the highly anthropophilic (human seeking), filarial parasite-transmitting vector from Guatemala, *S. ochraceum*, was an order of magnitude greater than that of other species examined. A 15.3 Kda protein that produced hyperemia in the test assay was isolated from a laboratory colonized species, *S. vittatum*. Protein sequence information was used to isolate a cDNA clone and recombinant protein was produced in a baculovirus expression system. Studies are underway to elucidate the mechanism of action of this vasoactive protein and to examine its' relationship to vector status.

Assays of SGE apyrase activity were conducted to examine the relative proportions of ADP/ATP degrading action in black fly salivary glands. In addition to quantitative differences, qualitative biochemical properties of this enzyme activity differed among species indicating that these SGE protein(s) have species-related structural variations in the Simuliidae.

Inhibition of blood coagulation for these pool-feeding insects may also be an important contributor to vector competence. Three different proteins were identified that target specific components in the coagulation cascade focused at the common final pathway. Two of these proteins prevent action of the prothrombinase complex by inhibiting both factor Xa and factor V, while the third SGE protein blocks the catalytic activity of thrombin. Differences in these activities among black fly species correlate with host preference and thus may indicate a role in vector competence.

23-042

SUPPRESSIVE EFFECTS OF *IXODES RICINUS* SALIVARY GLAND EXTRACT ON NONSPECIFIC ANTIVIRAL IMMUNITY

J. Kopecký

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Tick saliva has been shown to contain molecules with immunosuppressive activities. There is increasing evidence that the nonspecific suppression of host immunity by tick saliva is exploited by tick-borne pathogens, e.g. arboviruses. We demonstrated a suppressive effect of the salivary gland extract (SGE) derived from partially fed *Ixodes ricinus* females on two important nonspecific antiviral mechanisms: interferon and natural killer (NK) cells.

SGE decreased cytotoxic activity of nonstimulated and *in vivo* polyinosinic-polycytidylic acid (poly I:C)-stimulated NK cells by 31% and 28%, respectively. NK cells stimulated *in vitro* by lipopolysaccharide (LPS) were markedly inhibited (74% suppression), while concanavalin A (Con A)-stimulated NK activity was not affected by SGE.

SGE profoundly decreased the interferon production induced by polyI:C *in vitro* (94% suppression), whereas its effect on LPS-stimulated splenocytes was less pronounced (62% suppression). Con A-stimulated splenocytes were resistant to the inhibitory effect of SGE.

Treatment of P388D1 macrophage cell line by SGE accelerated replication of tick-borne encephalitis virus in these cells early after infection, probably due to inhibition of interferon induction.

23-041

A LECTIN IN SALIVARY GLANDS OF THE TICK *IXODES RICINUS*: DETECTION AND PARTIAL CHARACTERIZATIONL. Grubhoffer^{1,2} and E. Durnová²

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Lectins are a structurally diverse class of proteins, their only common features being the ability to bind carbohydrates specifically and reversibly. They act as mediators of cell recognition in a wide range of biological systems including those of vector-pathogen.

Hemagglutinating activity (HA) was demonstrated and characterized in *Ixodes ricinus* salivary gland (SG) extracts. The highest HA was observed using mouse red blood cells. In a hemagglutination inhibition test the SG HA showed binding affinity to N-Acetylneuramic acid, D-Fructose, L-Rhamnose, D-Trehalose and several glycoconjugates. Mouse polyclonal antibodies were raised against this HA fractions; they have been used to identify a SG protein with a molecular weight of 70 kDa. New SG lectin may play a role in transmission of the tick-borne encephalitis virus and borrelia spirochetes.

23-043

DEATH AND DIFFERENTIATION OF TRYPANOSOME INFECTIONS IN TSETSE

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Trypanosoma brucei rhodesiense undergoes complex morphological and biochemical changes during its life-cycle in insect (tsetse fly) and vertebrate hosts. Parasites alternate between proliferative and non-proliferative phases to co-ordinate establishment in and transition between different host environments. Transformation from bloodstream form to insect (procyclic) forms in tsetse is rarely successful, most infections fail to establish in the midgut and the parasites die. Those which evade death form a persistent immature infection in the midgut where they wait for the opportunity to mature and complete their life cycle - again very few infections successfully undergo maturation (migration to the salivary glands and differentiation to mammalian infective form) and complete their life cycle. It appears that death and differentiation are closely linked in this parasite as parasite death during establishment can be prevented by inhibition of a lectin in the fly midgut; continuous inhibition of this lectin also blocks maturation suggesting that the lectin is utilised by the parasite to provide a signal for differentiation. Recent results suggest that lectins may signal trypanosomes to activate a cell suicide pathway similar to the process of apoptosis (a form of programmed cell death previously described in metazoa) and that such a mechanism may operate to promote and maintain homeostasis and genetic stability within a population. Electron micrographs of lectin treated trypanosomes *in vitro* and *in vivo* reveal surface membrane vesiculation and migration of chromatin to the periphery of the nuclear membrane. Examination of the DNA of *in vitro* treated trypanosomes shows the characteristic fragmentation of DNA into oligonucleosome sized pieces. The response of parasites to external signals leading to apoptosis are probably as varied as those in metazoa.

23-044

FACTORS INVOLVED IN TSETSE FLY - TRIPANOSOME INTERACTIONS
E. Osir (Nairobi-Kenia)

ABSTRACT NOT RECEIVED

23-045

MOLECULAR ASPECTS OF INTERACTIONS BETWEEN ANOPHELES TESSELLATUS AND THE HUMAN MALARIA PARASITES PLASMODIUM VIVAX AND P. FALCIPARUM.
M.S. Ramasamy¹, K.A. Srikrishnaraj¹, R. Kulasekera¹, I.C. Wanniarachchi², R. Ramasamy²

¹Vector Biology and ²Molecular Biology Laboratories, Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka.

Rabbit antibodies to whole midgut tissue of *Anopheles tessellatus* ingested in an infective bloodmeal reduced the infectivity of *Plasmodium vivax* to the vector in a complement independent manner. Antibodies raised to midgut glycoproteins also mediated a transmission blocking effect, suggesting that glycoproteins of the midgut and of the peritrophic membrane play a role in ookinetes traversing the midgut.

Trypsin produced in the midgut of the vector influenced malaria parasite infectivity as shown by the effects of feeding trypsin inhibitors in an infective bloodmeal. Inhibiting midgut trypsin activity increased the infectivity of *P. vivax* and decreased the infectivity of *P. falciparum* to the vector. This probably reflects specific differences in the biology of the two human malaria parasites within the mosquito midgut. The times of ookinete formation, midgut penetration and the requirements for chitinase may be relevant factors that differ between *P. vivax* and *P. falciparum*.

23-046

VECTOR COMPETENCE MECHANISMS ASSOCIATED WITH TICK SALIVA

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Vector competence in tick-transmitted Lyme borreliosis is determined by both physiologic and ecologic mechanisms. Anti-complement activity in the saliva of vector ticks (*Ixodes scapularis*), but lacking in non-vector ticks like *Dermacentor variabilis* appears to protect *Borrelia burgdorferi*, the Lyme borreliosis agent, from complement-mediated killing in the tick's midgut. Another ixodid tick, *Amblyomma americanum* exhibited variable salivary anti-complement activity, but rarely feeds on infectious hosts, thereby avoiding infection. Salivary anti-complement activity may be important in protecting other tick-transmitted pathogens from immune killing within the vector.

23-047

THE SENSORY PHYSIOLOGY OF HOST-SEEKING, HOST-ACCEPTANCE, MATING AND OTHER BEHAVIOURS OF BLOOD-SUCKING ARTHROPODS
P. Guerin, P. Steullet, C. McMahon, J. Taneja, P. Guerenstein, T. Kröber, M. de Bruyne, S. Grenacher, M. Dougherty and P.A. Diehl
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Chemostimuli are employed by ectoparasitic arthropods to guide them in a variety of vital behaviours such as host seeking and acceptance, feeding, mating, oviposition and selection of a refuge. Volatile chemostimuli involved in host-seeking have been most extensively studied by us for ticks. Despite the limited number of olfactory receptor cells in wall-pore sensilla on the first leg-pair, ticks are well equipped for host odour perception with olfactory receptors for CO₂, sulfides, aliphatic aldehydes, aromatics, lactones and fatty acids. Studies on the behavioural responses of triatomines to host odours have taught us that although these insects mostly walk to hosts, they employ strategies for host location similar to those employed by male moths when flying upwind to a female in a pheromone plume. Gustatory stimuli govern a variety of responses. The behavioural responses of ticks to steer-hair and its chemical constituents clearly demonstrate the role for contact chemostimuli in host recognition and acceptance. Regular sampling of the substrate with sensilla on legs and mouth-parts precede attachment for a blood-meal, and chemoreceptors housed in these sensilla are sensitive to a range of skin products. Receptors for gustatory stimuli involved in the tick mating process are carried on the same appendages. In addition to these host-associated behaviours, this exposé will also treat the role of natural products in refuge selection by triatomines and oviposition in phlebotomes.

23-048

TICK SALIVARY SECRETIONS AT THE TICK-HOST INTERFACE

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The problems associated with prolonged attachment to the host necessitates that ticks produce and inject substances to counter host defense mechanisms. The saliva of engorging ticks is rich in bioactive components that includes an impressive variety of pharmacological properties. Factors identified in the saliva include a mostly proteinaceous cement to help anchor the mouthparts to the host, various enzymes and enzyme inhibitors, histamine agonists and antagonists, anticoagulants, antiplatelet factors and protein immunomodulating factors. Other factors include prostaglandins and a novel caireticulin. These factors are believed to be major reasons for the biological success of ticks and their ability in vector pathogenic organisms, a list that includes viruses, fungi, rickettsiae, bacteria and protozoa.

23-049

IMMUNOLOGY OF TICK-HOST INTERACTIONS (IXODIDAE)

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Most cytokine productions, cutaneous inflammation and polarization of the TH1 and TH2 lymphocyte responses will be considered for different strains of mice infested by different tick species. Tick counter measures suppressing host macrophage and T-Lymphocyte subset elaboration of cytokines and T-lymphocyte proliferation will be discussed. A tick salivary gland derived protein suppressive for host immune function will be described.

23-050

GLOBAL DISTRIBUTION OF *Aedes albopictus* AND ITS CONTINUING SPREAD AT THE APPROACH OF A NEW MILLENNIUM

A.B. Knudsen¹, Invited speaker, Symposium on *Aedes albopictus*, Carl J. Mitchell and Giancarlo Majori, Organizers
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Aedes albopictus, an important *Stegomyia* species is probably best known to the public and media by its fanciful nick name, the Asian "Tiger Mosquito". This colorful *Aedes* mosquito gained notoriety as an aggressive day time biting, nuisance mosquito in the early 1980's when it began its invasion of the Americas. Today we recognize that the saga of the continuing adaptation and spread of this species into new regions and countries around the globe is the most dramatic account of an introduction of a medically important insect species in the 19th century.

Aedes albopictus has now been reported from more than 53 countries, including at least 24 states or territories of the United States. It was introduced into Brazil independent of the USA in 1986 and is reported to be present in at least seven states and nearly 673 counties and municipalities. It has spread southward from the USA into Mexico, to the Dominican Republic in the Caribbean and more recently it has been reported in Guatemala and Honduras. In Europe, it is present in Italy in ten regions and 19 provinces, threatening other southern European countries. Its introduction into West Africa as reported by Nigeria indicates that it has already spread to five states. This paper discusses the continuing spread and importance of the mosquito which ranks second only to *Aedes aegypti* in its importance as a vector of dengue and dengue haemorrhagic fever.

23-051

PRESENT DISTRIBUTION OF *Aedes albopictus* IN ITALY: POSSIBILITIES OF CONTROL AND FURTHER SPREAD.

G. Majori¹ and R. Romi

Invited speaker, Symposium on *Aedes albopictus*, Carl J. Mitchell and Giancarlo Majori, Organizers.

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Since 1991, when the first breeding population of *Aedes albopictus* in Italy was reported (Dalla Pozza e Majori, 1992), efforts to control the spread of the species were undertaken. All the known infestations of the species were controlled by employing larvicides and adulticides, and the local spread was carefully monitored, so that mosquito populations could be seasonally strongly reduced but not eradicated. A surveillance system, integrated into the structure of the National Health System, was quickly activated. However before 1993 most of the infestation have been detected by passive reporting, while after that year have been detected by active research in the "at risk" sites of the different regions. At present stable colonies of *Ae. albopictus* are reported in 10 regions and 19 provinces of our country (January, 1996).

As the importation in Veneto region of *Ae. albopictus* eggs infested tires from Atlanta (USA) was proved in 1992 (Dalla Pozza et al., 1994). Since then attempt to get a national regulation about importation of used tires from "at risk" countries were made. However data from ISTAT (Istituto Nazionale di Statistica) show most of the used tires imported between 1990 and 1994 were coming from CEE or European countries (meanly less than 2% of the total was coming from unidentified countries, not necessarily infested by *Ae. albopictus*).

Since late 1991 regional and local rules were made to control the infestations in the tire deposits. Despite this fact, the internal trade of used tires from Veneto region has been the source of infestation of the other Italian regions. Infact most of the infestation in our country originated in deposit of used tires. As a new colonization of *Ae. albopictus* generally involve a few individuals, the population of the insect can reach a detectable density only after some seasons of breed. Probably, for this reason, also the infestation detected by active research in the last 2 years originate from tire loads purchased before 1992-93.

23-052

THE ROLE OF *AEDES ALBOPICTUS* (SKUSE) AS AN ARBOVIRUS VECTOR (DIPTERA: CULICIDAE)

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During the past two decades *Aedes albopictus* has extended its range, largely through the transport of drought-resistant eggs and/or aquatic stages in used tire casings, to parts of North, South, and Middle America, the Caribbean, Africa, Southern Europe, and some previously uninfested Pacific Islands. This remarkably rapid spread of a mosquito species is unprecedented during this century and has heightened concerns among public health and vector control officials because of the known and potential vector relationships of *Ae. albopictus* with several arboviruses of public health and veterinary importance. *Aedes albopictus* is an established vector of dengue (DEN) viruses in parts of its range, and is a competent vector for a wide variety of arboviruses under experimental conditions. In addition to DEN viruses, *Ae. albopictus* has been found naturally infected with Japanese encephalitis virus (in Asia) and with eastern equine encephalitis, Keystone, Potosi, and Tensaw viruses (in the USA). The results of field and laboratory investigations concerning the known and potential vector relationships of *Ae. albopictus* for approximately two dozen arboviruses will be summarized and discussed.

23-054

THE SITUATION OF *AEDES (STEGOMYIA) ALBOPICTUS* (SKUSE, 1894) IN BRAZIL

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The mosquito *Aedes albopictus* was detected for the first time in Brazil in May 1986 in the state of Rio de Janeiro (located in the southeast of the country), and soon afterwards was found in the states of Espírito Santo and Minas Gerais, also in the southeast.

Although *Aedes albopictus* in Brazil has the capacity to transmit dengue virus, there have not been any outbreaks of dengue in areas where this species is present in the absence of *Aedes (Stegomyia) aegypti* (Linnaeus, 1752)

In 1992, Serufo reported the isolation of virus from larvae of *Aedes albopictus* from the city of Campos Altos, state of Minas Gerais, where *Aedes aegypti* had not been detected. During this same period three cases of dengue were registered in this city. Subsequently, the Virology laboratory of the Evandro Chagas Institute contested these results because in tests done in their laboratory none of these patients was found with serology positive for dengue virus.

In counties where both species coexist, there are many reports of epidemics, but it has never been possible to isolate dengue virus from *Aedes albopictus* mosquitos during these outbreaks

Therefore, although it is possible, until now transmission of dengue by *Aedes albopictus* has not been proved in Brazil

Aedes albopictus is now found in 7 of the 27 Brazilian states, distributed mainly in the southeastern region (Sao Paulo, Minas Gerais, Espírito Santo and Rio de Janeiro) and the south (Parana), and also in Maranhao (northeast) and the Federal District of Brasília (midwestern region).

Aedes albopictus is found in 876 (42,6%) of the 2058 counties in these 7 states. In the southeastern region, the principal area of concentration. 800 (50,8%) of the 1573 counties are positive for this species. In 169 (19,3%) counties in the area infested by *Aedes albopictus*, it occurs in the same county as *Aedes aegypti*¹

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¹ Source: Fundação Nacional de Saúde (data up to September, 1995)

23-053

CONTROL OF *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE) AND PERSONAL PROTECTION

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Aedes albopictus larvae are susceptible to organophosphate (OP) and pyrethroid insecticides, insect growth regulators (IGRs), and *Bacillus thuringiensis israelensis*. IGRs are more toxic to larvae than pyrethroids and OPs. In Asia, resistance to DDT is widespread, though variable, and malathion tolerance/resistance is reported in North America. Delivery of larval controls to outdoor habitats is difficult but slow release formulations of methoprene and temephos in tire casings result in 100% larval mortality for 150 and 360 days, respectively. Adults are susceptible to OP, carbamate, and pyrethroid insecticides applied mainly by ground ULV. Cyclopoid copepods, predatory mosquitoes (*Toxorhynchites* spp.), protozoans, bacteria, fungi, and fire ants are potential biological control agents. Source reduction is effective if container collection and removal programs are extensive and ongoing. Small scale eradication of *Aedes albopictus* in the urban setting has been achieved by combining chemical control with strict source reduction measures. Personal protection methods comprise mosquito avoidance, the use of physical barriers, such as mosquito netting and/or permethrin-treated clothing, and repellents. Repellent products containing >12% deet (N,N-diethyl-3-methylbenzamide) provide protection from bites by *Aedes albopictus* for 6 hours or longer.

23-055

REPRODUCTIVE PHYSIOLOGY OF *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

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Aedes albopictus is in the process of colonizing subtropical and temperate climates around the globe. Since it represents a potential vector of various arboviruses we have initiated a comparative investigation of its reproductive physiology at two temperature settings, 32°C versus 17°C. The respective isotherms are assumed to approximate the possible range of continuous breeding for this species.

Developmental time from hatching until eclosion varies between 8 days (17°) and 25-50 days (32°). These temporal differences are also reflected in the corresponding body sizes. The range of female wing lengths is 2.9-3.3 mm when raised at the high temperature and 3.4-3.7 mm at the lower temperature. For males the range of wing length is 2.3-2.8 mm at 32°C and 2.7-3.2 mm at 17°C. The average protein, lipid, and glycogen content of newly eclosed females and males does not differ significantly between the two temperatures. When related to body size however, the caloric contents clearly increase in both sexes when reared at the higher temperature.

The effects of starvation or of sugar-feeding are followed in terms of survivorship curves and by quantitative analyses of glycogenesis and lipogenesis.

The volume of blood consumption on a human host and female fecundity unexpectedly do not correlate with body size, but appear to depend on environmental temperatures. Quantitative aspects of blood meal utilization for oogenesis will be presented together with the duration of the gonotrophic cycle under these different temperatures.

These data shall provide a picture of the adaptability of this species to various climatic conditions outside its original geographic occurrence and to better understand its colonisatory success.

Supported by the Swiss National Science Foundation.

23-056

UTILIZATION OF LOW ALTITUDE REMOTE SENSING IMAGERY TO DETECT LARVAL HABITATS OF

TIRE-BREEDING MOSQUITOES G. M. Beavers, F. W. Knapp¹

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Improperly stored vehicle tires serve as an excellent breeding habitat for several mosquito species that vector a number of human and animal diseases. The commercial trade of used tires is also considered to be responsible for the introduction and spread of several mosquito species including the Asian tiger mosquito, *Aedes albopictus* (Skuse). Improperly stored or illegally dumped tires are clearly a public health concern. Locating tire piles is currently time consuming, labor intensive and generally ineffective. Remote sensing, particularly the use of multispectral video imagery obtained from aircraft, may be a viable alternative for detecting tire piles. Measurements of the spectral reflectance of tires and various background land coverages were acquired in the range of 350-2500 nanometers (nm) at 2 and 4 nm bands using a portable spectroradiometer. The best spectral regions for differentiating tires from background coverages were selected for data collection. Video imagery of tire piles varying in size from approximately 50 tires to 3 million, and located in rural and urban environments were acquired at altitudes of approximately 150,300 and 500 meters above ground level at three different spectral wavelengths. Individual video frames containing the tire sites were then digitized for computerized image analysis and both supervised and unsupervised classification algorithms were used in the analyses. Results of these analyses will be discussed.

23-058

SUSCEPTIBILITY LEVELS OF ITALIAN *Aedes albopictus* AFTER 3 YEARS OF ADULT AND LARVAL CHEMICAL CONTROL.

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Field population of *Aedes albopictus*, collected in Liguria, Veneto and Emilia-Romagna regions, were tested for determining the susceptibility levels to the most common insecticides employed in larval and adult control. *Ae. albopictus* samples were collected respectively in 1) urban area of Genoa; 2) urban area of Padua; 3) Abano, a town in province of Padua; 4-5) Calderara di Reno, a town in province of Bologna.

Larval and adult susceptibility tests were carried out according to the standard WHO method (WHO/VBC/81.805 and 81.806), employing the suggested diagnostic concentrations (WHO, Tech. Rep. Series, 86.737).

Batches of third/early-fourth instar larvae were exposed to different concentrations of temephos (between 0.001 and 0.02 mg/l) and chlorpyrifos (0.0005-0.01 mg/l). Batches of adult females were exposed to diagnostic dosages of permethrin (0.25%), deltamethrin (0.025%) and propoxur (0.1%). Results were compared with the baseline data on insecticide susceptibility obtained in 1992 on *Ae. albopictus* population from Padua.

All larval and adult populations tested were found fully susceptible to the discriminant dosages of the 5 insecticides used. Temephos (0.02 mg/l) and chlorpyrifos (0.01 mg/l) gave 100% larval mortality. Deltamethrin and propoxur provided 100% adult mortality after one hour of exposure; permethrin provided 90-96% mortality after one hour and 100% mortality after 2 hours.

However, comparing the values of LD50 of the populations tested with the 1992 baseline data, a decrease in the susceptibility to the antilarval temephos and chlorpyrifos was observed in 2 of the populations studied.

23-057

DENGUE AND DENGUE HEMORRHAGIC FEVER (DHF) IN CHINA — VECTORS AND CONTROL

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Since the end of seventies this century, with two peak epidemics during 1979-1981 and 1986-1987, the reprevallence of dengue has swept the coast areas of southeastern China after some 30 years of ceasing. From 1978 to 1995, over 670 thousand cases have been found in Guangdong, Guangxi, Hainan and Taiwan Provinces, with 490 deaths. Although *Aedes aegypti* is the main vector to dengue, the Asia tiger mosquito *Aedes albopictus* has played an important part not to be ignored. It's believed that some 38 thousand cases might be the victims of *Ae. albopictus* during the period of 1978-1993.

Integrated management (IM) with community participation (CP) has been emphasized in vector control programmes. Since 1990 *Ae. aegypti* has been wholly swept from some of the towns and villages along the coast of Guangxi Province by the implementation of IM strategy including the release of culicivorous fish to the containers and the prompt elimination of the temporary gathered water. With the active participation of well-organized residents, and by the health education, the hostesses successfully managed their habitations, Breteau Index (BI) declined by 97.4% in 112 villages of Chengmai County, Hainan Prov. Organized and supported by the local authorities and Canada's International Development and Research Center (IDRC), it's now in planning and programming to eliminate *Ae. aegypti* from the whole Hainan Island in a few years.

23-059

OPERATIONAL APPLICATION OF VARIOUS IDENTIFICATION TECHNIC OF VECTORS AND PARASITES OF ONCHOCERCIASIS IN WEST AFRICA

Akpoboua L.K.B., Bissan Y¹., Toé L²., and Hougard J.-M³. Onchocerciasis Control Programme In West Africa, Ouagadougou, Burkina Faso - 1 Onchocerciasis Control Programme in West Africa, Ouagadougou, Burkina Faso - 2 Onchocerciasis Control Programme In West Africa, Bouaké, Côte d'Ivoire - 3 Onchocerciasis Control Programme in West Africa, Ouagadougou, Burkina Faso.

Technics using cytological method and morphological characteristics other than wing tuft colour have provided valuable information about the taxonomy and the dynamics of the *Simulium damnosum* flies. A more recent molecular biology technic using the heteroduplex analysis of DNA has allowed the identification of all stages from egg to adult of *Simulium damnosum* complex from small fragments (part of antenna, etc) leading to a more accurate estimate of the vector capacity of the various species and a better management of the resistance phenomenon by rotational use of larvicides.

In collaboration with the onchocerciasis control programme (OCP), the development of DNA probes specific to *Onchocerca volvulus* and *O. ochengi* and furthermore to savanna and forest strains of *O. volvulus* by Dr Unasch from the University of Alabama in Birmingham has been a major contribution in the assessment of the transmission due only to *O. volvulus*. As part of this collaboration, the same author recently developed the technic of blackflies pools screening by polymerase chain reaction (PCR) for the infection prevalence assessment. This new technic will help in the detection of any recrudescence of infection in oncho free zones.

23-060

MOLECULAR APPROACHES TO THE DIAGNOSIS OF ACARICIDE RESISTANCE IN THE CATTLE TICK *BOOPHILUS MICROPLUS*

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Since the cattle tick *Boophilus microplus* was introduced into Northern Australia in 1872, it has extended in distribution into most tropical and sub-tropical high rainfall areas and has developed resistance to a broad spectrum of acaricides. The continued use of a limited number of available acaricides is necessary to sustain efficient cattle production. Current methods for the detection of acaricide resistance take 6 to 15 weeks. Resistant ticks remain uncontrolled and may be dispersed during this period. The existing tests are not readily applicable to some new acaricides (c.g. growth regulators and systemics). We have examined alternative methods for the detection of resistant ticks using DNA technology with the aim of developing more rapid tests suitable for all classes of acaricide. The arbitrary primed polymerase chain reaction (AP-PCR) was used to analyse DNA from resistant and susceptible strains. The results demonstrated that the DNA fragments generated were similar for all strains tested when 20 different primer pairs were used. Using a different approach, we have now cloned and sequenced several tick microsatellite loci. Some loci contain extensive adjoining dinucleotide repeats of various types. PCR amplification of these loci from different tick strains is currently under evaluation as a means of generating strain-specific DNA fingerprints. It is possible that DNA fingerprinting could be used for the identification of resistant *B. microplus* strains and their origin worldwide.

23-062

RELAY TRANSMISSION OF MALARIA IN MALI.

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In the village of Donéguebougou a longitudinal entomological study was conducted from June 1994 to May 1995 to assess the malaria transmission pattern. The vectors were collected by spray and landing catches and identified by morphology, chromosomal and/or PCR methods. The infection rates were determined by ELISA. The malaria vector population was composed of 63% of *An.gambiae s.s.*, 26.4% of *An.arabiensis* and 10.6% of *An.funestus*. *An.gambiae s.s.* predominated during the rainy (June-October), *An.funestus* in the dry cool (Dec-Feb) and *An.arabiensis* in the dry hot (March-May) season. The infection rates were 5.4% for *An.gambiae s.s.*, 7.7% for *An.arabiensis* and 6.0% for *An.funestus*. Total annual entomological inoculation rate (EIR) was 32.5: 19.2 for *An.gambiae s.s.*, 10.4 for *An.arabiensis* and 2.9 for *An.funestus*.

Within *An.gambiae s.s.* the three chromosomal forms found were 16.1% Bamako, 20.6% Savanna and 56% Mopti and 7.3% others. Bamako and Savanna were seen only during the rainy season while Mopti persisted throughout the year. The total annual EIR for *An.gambiae s.s.* was partitioned as follows: 19.6% Bamako, 25.9% Savanna, 35.4% Mopti and 19.0% others. Transmission occurred throughout the year in close association with the population dynamics of the vector species and the chromosomal forms of *An.gambiae* in a relay pattern as species and chromosomal forms sequentially predominated at different times of the year.

23-061

MOLECULAR EVOLUTION AND SPECIATION IN *PHLEBOTOMUS PAPATASI* AND *LUTZOMYIA WHITMANI* (DIPTERA: PSYCHODIDAE) IN RELATION TO PERIDOMESTIC TRANSMISSION OF *LEISHMANIA*Paul Ready, John Day, Selma Esseghir¹, Jennifer Sackin, Riadh Ben-Ismaïl¹, Clive Davies²

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We have assessed the relationships among geographical populations of two phlebotomine sandfly species by comparative analysis of mitochondrial and nuclear DNA sequences. *Phlebotomus (Phlebotomus) papatasi* (Scopoli, 1786) has a wide geographical distribution, from the western Mediterranean Basin to the Indian subcontinent. Peridomestic populations are found throughout this species' range but transmission of *Leishmania major*, although widespread, is limited to regions occupied by the parasite's reservoir hosts, which are usually gerbillid rodents. For *P. papatasi*, we have tested the hypothesis that peridomestic populations do not represent one or more sibling species distinct from populations associated with gerbil colonies.

In contrast, the neotropical phlebotomine we are investigating exhibits marked geographical differences in vectorial roles. *Lutzomyia (Nyssomyia) whitmani* (Antunes & Coutinho, 1939) is an incriminated vector of *Leishmania braziliensis* in North East and South East Brasil, where it is synanthropic, but of *Leishmania shawi* in Amazonia. In this case, we are testing the hypothesis that the Amazonian populations represent a distinct phylogenetic and biological species which is not abundant peridomestically.

This work is supported by project grants from the CEC/EU (Brussels) and The Wellcome Trust (London).

23-063

DISCRIMINATION BETWEEN *ANOPHELES GAMBIAE* S.S. CHROMOSOMAL FORMS BY PCR-RFLP ANALYSISG Favia, C.Fanello, Y T Touré¹, N Sagnon², M Coluzzi, A della Torre

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The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach has been used to discriminate between two chromosomal forms of *Anopheles gambiae* sensu stricto, the major malaria vector in Africa. These forms, namely Savanna and Mopti, are morphologically indistinguishable, but characterized by specific inversion polymorphisms. A ribosomal DNA fragment of 1.3 kilobase, that contains part of the Intergenic Spacer (IGS), has been PCR-amplified from genomic DNA extracted from pools of individuals belonging to either a Savanna or a Mopti laboratory colony. The fragment obtained was cut with several restriction enzymes, two of which (Tru9I and HhaI) yielded specific patterns for each colony. Carnoy's preserved field-collected, sympatric specimens of Mopti and Savanna from different localities in Mali and Burkina Faso were karyotyped and then analyzed by the above procedure: they were shown to correspond to the expected pattern. Therefore the PCR-RFLP technique may be applied for a unambiguous identification of the two *An.gambiae* forms and used to analyze large field-collected samples with no constraints due to life stages, sex or storing conditions. The analysis of the sequences of the PCR fragments is in progress in order to design specific diagnostic primers to be used to eliminate the restriction step from the procedure.

23-064

INTER SPECIES COMPARISONS OF REPETITIVE DNA COPY AND SEQUENCE VARIATION IN MEMBERS OF THE *ANOPHELES CULICIFACIES* COMPLEX

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Members of the *Anopheles culicifacies* complex are the major vectors of malaria on the Indian sub-continent. Four sibling species have been identified which can only be distinguished by analysis of ovarian nurse cell polytene chromosomes. In contrast to several other mosquito species complexes, the development of an alternative method for species identification based on DNA probes has proved difficult. Our studies using the standard approach, involving differential screening of small genomic DNA libraries have identified a number of DNA fragments which represent moderate to highly repetitive sequences. These fragments show differences in the degree of hybridisation to DNA from member species, but none were species specific.

A closer examination of the isolated DNA clones and homologous regions within the different species was made in the hope of fine tuning the species specificity. Details of five clones will be presented four from species B (B113, Ac9, Ac10, and Ac11) and one from species C (Ac3). Clone Ac11 contains an almost complete ribosomal RNA gene repeat unit, which has been fully mapped. Clone B113 contains a ~600 bp subfragment, adjacent to non-repetitive DNA. The sub-fragment has little or no internal repetition and appears to be a dispersed repeat. The remaining three clones contain fragments of satellite DNA sequences and are composed of tandem repeats.

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23-065

VARIATION IN rDNA, mtDNA AND RAPD IN THE *ANOPHELES CULICIFACIES* COMPLEX (DIPTERA: CULICIDAE)

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Anopheles culicifacies is known to be a species complex that includes the most important malaria vectors in the Middle East and Indian sub-continent. Based on sequence differences in the second internal transcribed spacer (ITS2) of the ribosomal DNA (rDNA), we have developed a diagnostic Polymerase Chain Reaction (PCR) assay which readily distinguishes species A and B over an extensive part of their geographical range.

Significant sequence differences between these species have also been found in the mitochondrial DNA (mtDNA) cytochrome oxidase I-II genes, as well as some sequence variation between different populations within species. Species-specific mtDNA haplotypes may be distinguished both by sequencing and by restriction mapping.

Species-specific differences in the complex can also be demonstrated using random amplified polymorphic DNA (RAPD) PCR. In addition, some RAPD primers show variation between individuals from different geographic localities. Species-specific fingerprints are obtained using a novel technique involving PCR primers which carry 3'-anchored dinucleotide repeats.

We discuss the significance of our findings for understanding of the *An. culicifacies* complex and its role in malaria transmission. We also highlight some of the needs for future research.

23-066

BIOLOGY AND BEHAVIOR OF FORENSICALLY IMPORTANT FLIES

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BBC Natural History has released a forensic biology film called "Creatures in Crime" or "The Witness Was a Fly." We present the entomological portions with footage, often dramatic, of the biology and life cycles of some forensically important flies, including *Chrysomya rufifacies*, *Cochliomyia macellaria*, *Phormia regina*, and *Piophilidae casei*. One sequence shows the catapulting behavior of postfeeding larvae of *P. casei*, captured possibly for the first time by high speed camera. Another shows the predatory behavior of third instar larvae of *C. rufifacies*. Homicide cases are presented including those of Dr. Zakariah Ercinçlioglu of Cambridge University.

23-067

EFFECTS OF BURIAL ON RATE OF DECOMPOSITION, TEMPERATURE, AND INSECT SUCCESSION ON CARRION IN TWO BIOGEOCLIMATIC ZONES IN BRITISH COLUMBIA, AND THE IMPLICATIONS FOR FORENSIC ENTOMOLOGY.

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The time of carrion colonization by insects can be used to accurately determine time of death in homicide victims. However, there is very little knowledge on insect succession on buried carcasses, despite the fact that many homicide victims are buried. Until this work, no data on buried carcasses were available in Canada. Pig carcasses as human models were buried in Interior and Lower Mainland British Columbia, and were exhumed at regular intervals over a one year period and compared with above ground carcasses. Results of the effect of burial on insect colonization, rate of decomposition and carcass temperature, will be presented and the implications for minimum post mortem interval determinations will be discussed.

23-068

EXTRACTION, AMPLIFICATION, AND SEQUENCING OF HUMAN MITOCHONDRIAL DNA FROM INSECTS OF FORENSIC IMPORTANCE

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The ability to identify individual human hosts based on analyses of blood recovered from the digestive tract of hematophagous arthropods has been a long term pursuit in both medical and forensic entomology. Bloodmeal individualization techniques can bring important advancements to studies of vector-borne disease epidemiology. Forensically, these analyses may aid in assailant identification in violent crime cases where blood-feeding insects or their excreta are recovered from victims or at crime scenes. Herein, we report the successful isolation, amplification, and sequencing of human mitochondrial DNA obtained from adult human crab lice fed on human volunteers. Adult lice were removed from recruited volunteers frequenting inner city health clinics. Live lice were killed by freezing and subsequently air dried at ambient temperature. A reference saliva sample was obtained from each volunteer and served as a DNA comparison standard. Volunteers were afforded free, approved pediculosis treatment. Individual lice were subsequently processed using procedures developed for the extraction of mitochondrial DNA from human hair and bone. The resulting DNA was amplified by the polymerase chain reaction and sequenced by automation. While preliminary in nature, our results point to valuable avenues for future entomological research.

23-070

FORENSIC ENTOMOTOXICOLOGY - AN UPDATE.

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Until some years ago diptera larvae were used in forensic science only to provide useful information on Postmortem Interval, on previous location of the corpse or its geographic origin. Entomotoxicology is a relatively new branch in forensic entomology due to the unbelievable increase in drug-related deaths all over the world. Recent studies have revealed that Diptera larvae can be used as toxicological specimens when no other cadaveric substances are available. So the analysis of Diptera larvae grown on a corpse can give useful indications regarding previous intoxication of the body (phenobarbital, triazolam, oxazepam, levomepromazine, cocaine, heroin, etc.). A toxicological cause of death can be now revealed even in the absence of the body just analyzing the larvae fed on it. Furthermore most of the chemicals can influence the life-cycle of maggots; recent studies concerning the effects of drugs and toxins on the rate of larval development have confirmed the opportunity of using larvae recovered from a corpse for PMI.

23-069

COPING WITH THE INHERENT VARIABILITY IN CARRION INSECT GROWTH AND SUCCESSION: STATISTICAL TECHNIQUES FOR ENTOMOLOGICALLY-BASED ESTIMATION OF THE POSTMORTEM INTERVAL

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We are developing techniques for calculating the statistical uncertainty in estimation of the postmortem interval (PMI). For PMI estimations based upon the length or weight of a larva from the victim, we suggest a computationally intensive but relatively straightforward method for calculating the regression line and a confidence interval about the estimated larval age. For PMI estimations based upon the stage of succession, we have expanded the occurrence matrix approach of Schoenly *et al.* for use with replicated data. A probability distribution for all possible sample combinations (each taxon or stage either present or absent) for each day of succession is empirically estimated. An adaptation of Fisher's exact test is then used to calculate a p-value for observing a particular sample at a given point of time in succession. The result is an interval of likely PMI values for a given death scene sample.

23-071

STATE OF OKLAHOMA v. HOWELL (PART I): THE USE AND VALUE OF ENTOMOLOGICAL EVIDENCE DURING THE MURDER INVESTIGATION AND SUBSEQUENT PRE-TRIAL IN OKLAHOMA

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A bloated decomposing corpse of a human male was found under a pile of household items (comforter, dresser drawers, television set, cardboard and paper) beside a residential driveway in Stroud, Oklahoma, in the late afternoon on August 8, 1994. The last time the victim had been seen, he and his wife were leaving a nightclub at about midnight on August 4 and were engaged in a bitter argument. When questioned, the wife claimed that she had last seen her husband during the late evening of August 6, when he left with one of her friends.

Entomological evidence was collected from the remains at the scene and at autopsy. Two species of Calliphoridae were present as larvae. The oldest immatures were late, non-migrating 3rd instars *Cochliomyia macellaria* (F.); also present were early 3rd instar *Phormia regina* (Meigen). Under the climatic conditions prevailing during the period in question (highs about 30°C) and the exposure of the remains to direct solar radiation (no shading until late afternoon), it was estimated that in this case, oviposition of the fly eggs producing the larvae collected must have occurred no later than mid-morning on August 5. The prosecutor's insect evidence thus appeared to refute the wife's alibi. The defendant was charged with first-degree murder and the case was scheduled for trial, whereupon the defense requested a hearing regarding admissibility of the entomological evidence. When the evidence was ruled admissible, defendant sought a plea agreement and accepted the 25-year sentence offered by the prosecution. The importance of the insect evidence in this case is underscored by the fact that a lengthy and expensive trial was avoided.

23-072

STATE OF OKLAHOMA V. HOWELL (PART II): PROCEDURAL AND OTHER ISSUES IN RELATION TO ADMISSIBILITY AND WEIGHT OF ENTOMOLOGICAL EVIDENCE IN A MURDER TRIAL.

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To affect the result at trial in U.S. state and federal jurisdictions, scientific evidence, including entomology, must be admitted by the court. Motions to exclude may be made before or during trial and if successful, the evidence will not be considered. Common theories under which such exclusionary motions are made include logical and legal relevance, or competency of the evidence based either on privilege or on trustworthiness. The abovestyled case occurred in 1994 before Oklahoma adopted the admissibility standards articulated in *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 113 S.Ct. 2786 (1993), but was tried after such were adopted in *Taylor v. Oklahoma*, 889 P.2d 319 (1995). The defense introduced a pretrial motion to exclude that raised the issue of admissibility under 12 Okla. St. § 2702 (Oklahoma's codification of Federal Rule of Evidence 702) and requested a preliminary hearing under the *Frye* "generally accepted science" standard. The court applied *Daubert* and ruled the evidence admissible. The defense at this point had both consulting and testifying experts and continued matching the facts of the case with those strategies that might affect the weight of the scientific evidence in the prospective jury's evaluation. Although defendant pled guilty to a lesser charge before the case went to trial, *Oklahoma v. Howell* affords opportunity to evaluate the adversarial tactics that are commonly employed in defending against scientific testimony. Here, there were both strong and weak aspects to the prosecution's entomological case and the defense was prepared to exploit each of these on the merits before impeaching the prosecution's expert. The discussion will focus on the potential impact of the *Daubert* ruling on entomological testimony in light of the Federal Rules of Evidence, state codifications thereof, and the ambiguities inherent in determination of what constitutes "good science." Additional points include those entomological aspects in controversy in the instant case and how they could be attacked in the contexts of both court-appointed and adversarial expert witnesses.

23-074

PATTERN OF MYIASIS IN HUMAN CADAVER AND THE ESTIMATION TIME OF DEATH.

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A total of over 20 cases of myiasis found in exposed corpses for forensic investigations have been sent to and recorded in the Department of Parasitology. In all of these cases early stages of maggots were allowed to develop into adult stage in the laboratory and both were later identified. The dipterans involved are *Chrysomya megacephala*, *C. rufifacies*, *Sarcophaga*, *Hermetia illucens* and *Synthesiomyia nudiseta*.

The estimation of time of deaths for these forensic cases could be derived from the species and stage of dipterans found *in situ*.

In most cases the corpses attract primary and secondary invaders. The ages of the larvae and species of arthropods attacking the corpses can help forensic experts to determine the time of death, provided the detailed life cycle and history of the dipterans are known.

23-073

THE USE OF CARRION AS BREEDING SITES BY THE SHEEP BLOWFLY *LUCILIA SERICATA* AND OTHER CALLIPHORIDAE.

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The use of vertebrate carcasses as breeding sites by larval Diptera was examined on two farms in South West England. *Calliphora vicina* was the dominant species to emerge; 19,294 individuals emerged from 175 of the 241 infested carcasses recovered. *Lucilia sericata* emerged from only 39 of the infested carcasses, at a median of ten adults per infested carcass. Other species of *Lucilia* present were *Lucilia ampullacea*, *Lucilia caesar* and *Lucilia illustris*. The highest number of *L. sericata* emerged from carcasses placed in open pasture, the highest number of *C. vicina* emerged from carcasses in hedgerow, while the highest numbers of *L. caesar*, *L. ampullacea* and *L. illustris* emerged from carcasses in woodland. The duration of exposure of carcasses in the field was negatively related to the size of the adult *L. sericata* which emerged and, in the woodland and hedgerow habitats, to the number of *L. sericata* which emerged. These data indicate that the larvae of *L. sericata* in carcasses experience significant levels of competition and that the intensity of this competition may be sufficient to reduce the number of *L. sericata* that are able to emerge from carrion. The results are discussed in relation to the development of novel methods of control of blowfly strike of sheep.

23-075

THE DEVELOPMENT OF LARVAE OF *CALLIPHORA ERYTHROCEPHALA* IN A BODY BURIED IN THE SNOW

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The development of fly larvae exposed to low temperatures has been a subject of interest for some time, also due to the practical implications which derive from it. Unfortunately it has not yet been possible to obtain results of any absolute certainty and, for this reason, this case seemed to us worthy of note. It is the case of the discovery of *calliphora erythrocephala* in a body of the female sex, found in the open countryside after the thawing of a heavy snowfall which had lain for more than 15 days in the month of January, with low night temperatures but with good exposure to the sun during the day. The reexamination of the data contained in literature together with the experimental studies allowed the time of death to be established before the snowfall, a finding which was also in agreement with the circumstantial data.

In fact, it was possible to establish that the laying of the eggs had taken place before the snowfall and the larvae had developed despite adverse environmental conditions.

23-076

EXTERNAL TEMPERATURE AND BODY TEMPERATURE IN LARVAL INFESTATION, A RELATIONSHIP.

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In forensic cases maximum and minimum temperature at the crime scene is recorded for entomological investigations. Temperature is indeed the most important variable for the rate of growth of fly larvae found in a corpse. Previous studies have discussed the effect of microclimatic conditions on larval development. An increase of internal temperature of a corpse during the advanced stages of larval infestation has been supposed; this over-heat has been related with the metabolic effect of larvae feeding clusters on the body. Anyway no relationship has been described between temperature at which the body was exposed and internal temperature in case of larval infestation. In such a case, time of death determination by internal body temperature can be underestimated. We have then performed an experimental investigation on animal corpse (rabbit) to study the autonomous capability of the larval cluster to increase the internal temperature of a corpse. Data concerning environmental microclimatic conditions (max. and min. temperature, humidity) at the body exposure place and inside body temperature in cluster feeding site were recorded daily. The results revealed a different increase of internal body temperature in cluster feeding site related with the larval developmental rate, the site of the cluster and the external microclimatic conditions.

23-078

THE USE OF DIPTEROUS MAGGOTS AS TOXICOLOGICAL INDICATORS IN FORENSIC ENTOMOLOGY

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Forensic Entomology is a relatively new research field in South Africa. Estimating the post mortem interval (PMI) is probably the most important aspect of the Forensic Entomologist's task. Although the development stage of the insect larvae (dipterous maggots) on the corpse may be influenced by environmental conditions, drugs or poisons in the corpse may also effect its development and thus have an influence on the estimation of the PMI. In South Africa there is an escalating misuse of clozapine in combination with alcohol to incapacitate victims before robbery. Although many of the victims are brought to hospital for treatment, the number of victims which die as a result of the assault is increasing. Very often the bodies are found several days after death, at a stage when the usual toxicological determinations are complicated by decomposed tissue. This prompted us to establish whether dipterous maggots can be used as toxicological indicators in forensic entomology case studies.

A headspace GLC procedure was developed for the assay of ethanol in the maggots and in the stomach contents, muscle and liver tissue of decomposing rabbit carcasses. Using 20 mg tissue (or maggots) per headspace vial, the assay is sensitive enough to determine ethanol at 0,4 µg contained per headspace vial which converts to 20 mg/100 g tissue (or maggots). After 17 days, when the last maggots left the rabbit carcass, ethanol could still be detected in the maggots.

The assays on the combination of ethanol and clozapine is still in progress.

23-077

THE DETECTION OF DRUGS IN INSECTS AS ALTERNATIVE TOXICOLOGICAL SPECIMENS FOR FORENSICS

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The forensic importance of insects and their remnants has been well established in the determination of time of death, and has recently been recognized as an alternative toxicological resource. The development of methods of analysis for toxins and controlled substances in insects can make an important contribution to the assessment of cause/manner of death in cases of advanced decomposition. Toxicological samples are often absent when mummified/skeletalized remains are found and entomological samples may provide the only clues. Applicable methods have been developed for drugs in beetle exuviae, fly larvae and puparia. Anti-depressants and stimulants in insects have been quantified using hyphenated techniques of chromatography and mass spectrometry. These methods have been utilized in actual forensic casework and provided complementary information. In addition, controlled laboratory animal studies are being conducted to examine the transfer and pharmacology of drugs from donor subjects (rabbit liver) to carrion insects. These experiments demonstrate that amounts found in insects are related to the dose and time of death after administration of the drug. Both parent and metabolite drugs can often be found in the insect tissues.

23-079

OPIATE ANALYSIS ON EMPTY PUPARIA - POSITIVE RESULTS

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The contribute of entomological evidence in forensic cases is already known for evaluating the time and geographical location of the body. Fly larvae, reared on a known toxicological substrate, have been also tested for drug qualitative analysis. So it has been proved that cadaveric fly larvae can be used as laboratory specimens when no other cadaveric remains are available. Anyway this is a very rare case in criminal investigation. More easy to find in the daily forensic routine skeletonized remains with empty puparia. In these cases toxicological analysis can be performed only on bone fragment. We have than set up an experimental research to reveal if toxicological analysis can be performed with success on empty puparia. Empty cases of *Calliphora Erythrocephala* obtained by 20 experimental liver rearings with known different morphine concentration. Opiate quantitative and qualitative analysis was then performed using Fluorescence Polarization ImmunoAssay (FPIA). The results revealed an opiate positive qualitative analysis for empty cases obtained by the specimens with the highest morphine concentration used (10 mcg/g tissue). This evidence allowed to use in extreme forensic cases even the empty puparia of cadaveric blowflies as useful specimens for toxicological analysis. A new field in forensic entomotoxicological is opened. In the future when all the toxicological research will be performed, a complete drug screen will be allowed even in historical cases just using as toxicological specimen the empty cases of cadaveric flies.

23-080

FORENSIC ENTOMOLOGY ACTIVITY IN SOUTHERN ITALY: AN ENTOMOLOGICAL STUDY ON ANIMAL CORPSES.

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In forensic entomology any accurate determination of Postmortem Interval by arthropod succession should be done using species related with the place where the body was found. The goal of this research is to provide a more reliable method for PMI estimation based on entomological evidence and seasonal factors revealed all over the year in Southern Italy. For this purpose we have performed an experimental investigation on the insect fauna of decaying corpse carried out on animal carrion (rabbits) placed at intervals of about two weeks in open field in a suburban habitat. We have intentionally limited our researches to the study of cadaveric flies of the first wave for a better definition of the time since death in the earliest stages of decomposition. Data concerning climatic conditions, body decomposition and insect activity have been recorded daily. Entomological specimens from corpse and from surrounding area before and after removal of the remains have been collected. We have indeed analyzed the time necessary for egg deposition, egg hatching, larvae development and pupation of blowflies based on different parameters such as degree of decay and seasonal weather (lighting, temperature, humidity, rainfall).

23-081

HANGING OUT AT THE SIXTEENTH HOLE AND JACK'S IN THE BOX

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Patterns of invasion of remains by arthropods and resulting patterns of decomposition may be altered by the circumstances of the body. Two examples of unusual disposals of remains are presented here with comments on the changes in the resulting patterns of arthropod invasion.

In the first instance, the body was found hanging in a wooded area of the Ala Wai Golf Course in Honolulu. In this case, the initial invasion was by arthropods which could fly onto the body. Access by soil dwelling arthropods was delayed until the body stretched enough for the feet to reach the ground. There were significantly fewer taxa involved in this decomposition when compared to remains exposed on the ground surface. A secondary site of arthropod activity was noted in the drip zone under the suspended body.

In the second instance, the body had been dismembered and placed inside a metal tool box. When discovered, there were few arthropod taxa represented by living forms or puparial cases. The postmortem interval was established by analyses of the activities of the Stratiomyidae *Hemelia illucens* and the period required for a colony of ants to produce reproductive forms.

23-082

INTRODUCTION TO THE CONCEPT OF REPLACEMENT OF A VECTOR POPULATION BY A HARMLESS GENOTYPE

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Members of an insect species which is normally a vector of human disease could be rendered harmless by a gene or genes which caused either (i) interference with development of the parasite (refractoriness) or (ii) biting of animals (zoophily) and not humans.

For efficient spread in a wild population it is desirable that a single gene (not polygenes) is involved and that it is dominant and does not cause serious loss of fitness. Two or more independent forms of refractoriness would make it less likely that the parasites could evolve evasion mechanisms.

A suitable genetic driving system could (i) spread the genes from a small "seeding", and (ii) counter the effects of immigration from surrounding vector populations. These are potential advantages of making populations genetically harmless as compared to attempted eradication by the mass release of sterile males.

23-083

IDENTIFICATION OF GENES THAT CONFER PLASMODIUM-REFRACTORINESS IN ANOPHELES GAMBIAE

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One approach to the identification of genes that could reduce or block the ability of the mosquito *Anopheles gambiae* to transmit malarial parasites has been to genetically select strains of this mosquito that are refractory to the parasite and then use molecular approaches to identify the responsible genes.

Two forms of refractoriness which seem to be regulated by only one or a few genes have been identified by this approach. In one form of refractoriness, the ookinete is encapsulated shortly after it completes its passage through the midgut epithelium. In the other form, the parasite is killed by lysis as it passes through the midgut cells. A strategy for positional cloning of these encapsulation and lytic factor genes is now being implemented. This strategy combines high resolution genetic mapping with microsatellite loci and chromosome walking with a large fragment genomic library in a bacterial artificial chromosome (BAC) vector. Ultimately, candidate genes identified by this strategy will be verified by germ line transformation. Progress in the development of these tools and their use in cloning malarial parasite refractoriness genes will be described.

23-084

THE INTRODUCTION OF GENES CODING FOR TRANSMISSION BLOCKING ANTIBODIES INTO THE GENOME OF *ANOPHELES GAMBIAE*: A PROGRESS REPORT

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Insect vector control has proved an effective method for reducing the transmission of disease-causing organisms to human populations in many tropical countries. However, the development of insecticide resistance by vector insects, the cost of developing and registering new insecticidal compounds, and the increase in legislation to combat the detrimental effects of insecticidal residues on the environment, have emphasised the need to assess a variety of alternatives to vector control. We are interested in employing direct genetic manipulation of insect vector genomes either to suppress vector populations, or to alter their ability to transmit disease-causing organisms in such a way as to have a profound and long-lasting effect on disease transmission. The particular focus of our research is to alter the ability of mosquitoes to act as vectors of malaria by introducing heterologous transmission modulating genes into the mosquito genome. In a series of model experiments in which we will assess this concept, we have introduced the genes necessary to produce transmission blocking antibodies in the mosquito midgut so that they are expressed when it takes an infected blood meal. Transmission blocking antibodies, based on the Pfs25 and Pbs21 antigens of *Plasmodium falciparum* and *P. berghei*, are well characterised molecules known to block transmission when present in an infected blood meal. The progress which has been made towards achieving this goal will be discussed, with particular emphasis on: a) the identification of endogenous transposable elements in mosquitoes which may be used as DNA vectors for the manipulation of mosquito genomes; b) the identification of putative gut-specific, blood-meal inducible promoters in mosquitoes which may be used as a transmission-blocking antibody expression cassette; c) the assessment of the activity of these promoters in mosquito tissues and cells.

23-086

WOLBACHIA INFECTIONS AS A GENERAL VEHICLE TO DRIVE REFRACTORINESS GENES INTO INSECT POPULATIONS

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Wolbachia infections could potentially be used as vehicles to drive refractoriness genes into natural populations of insects. This could be accomplished in two ways depending on the mode of inheritance of the genes of interest. Natural *Wolbachia* infections would indirectly drive maternally inherited genes with them as they spread into a population through the action of cytoplasmic incompatibility. Experimental data shows that *Wolbachia* superinfections provide a method to repeatedly spread maternally inherited genes into the same host population.

Modeling shows that chromosomal genes could also be spread into an interbreeding population if the *Wolbachia* genes that induce cytoplasmic incompatibility could be isolated from *Wolbachia*, integrated onto insect chromosomes and expressed appropriately. As these genes spread they would be expected to drag with them linked genes of interest.

23-085

TRANSPOSONS AS GENE DRIVING MECHANISMS

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ABSTRACT NOT RECEIVED

23-087

NATURAL POPULATION REPLACEMENT AND COMPETITIVE EXCLUSION IN THE *ANOPHELES GAMBIAE* COMPLEX

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The severity of the malaria problem in sub-Saharan Africa depends mainly on the exceptionally high vectorial capacity of the two most anthropophilic members of the *Anopheles gambiae* complex, namely *An. gambiae* and *An. arabiensis*. These mosquitoes represent the principal target for the development of new control strategies including vector population replacement whose feasibility needs to be evaluated. Within this context, some of the available biogeographical data are of particular interest since they suggest the natural occurrence of replacement and competitive exclusion. The most relevant case refers to the sub-Saharan belt of arid savannas, below the 800-mm isohyets, from Senegal to Sudan. *An. arabiensis* alone exploits this environment in Sudan, successfully colonizing the seasonal, rain dependent, larval breeding sites as well as those available during the dry season, mostly associated with irrigation (e.g., Gezira). *An. arabiensis* coexists westwards with the chromosomal form Savanna of *An. gambiae*, the former sibling being still generally dominant in most localities of Cameroon, Nigeria and Senegal. The picture changes completely in Mali and Burkina Faso where the irrigated areas show the unexpected dominance of *An. gambiae* represented by its chromosomal form Mopti, a very effective dry season breeder typical of rice cultivated zones. However, out of the Mopti range the same ecological areas are successfully colonized by *An. arabiensis* and, less intensively, by *An. gambiae* Savanna. Incidentally, *An. arabiensis* is well known as the dominant member of the complex also in rice cultivated areas of East Africa. We favour the hypothesis that, wherever dry season breeding opportunities exist in Mali and Burkina Faso, the most recent Mopti taxon replaced *An. arabiensis* and *An. gambiae* Savanna. These taxa occur only at the periphery of the Mopti colonized areas and the stability of such pattern of distribution during the rainy season suggests the existence of competitive exclusion.

23-088

**A SPATIAL MODEL FOR GENETICALLY MODIFYING
FLUCTUATING MOSQUITO POPULATIONS**

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Our stochastic automaton model for genetically modifying anophelines in nature represents transposon release into a dispersed, clumped, seasonally fluctuating, locally mating population. Contrasting scenarios of population fluctuation are compared: one assuming that anopheline density remains constant; another assuming rigorous estivation and the last assuming seasonally reduced breeding density. Diverse fitness parameters and transposition efficiencies also are examined. As transposon fitness approaches 50%, fixation requires that insertion efficiency approaches unity. In a heterogeneous space and with fluctuating population density, however, fixation may require thousands of generations. When estivation is stringent, fitness must exceed 70% to achieve fixation. We conclude that the clumped, fluctuating characteristics of peridomestic anopheline populations resist fixation.

23-089

BIOLOGICAL CONTROL OF *CULEX QUINQUEFASCIATUS* BY MYCELIA AND METABOLITES OF *PAECILOMYCES LILACINUS*

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Paecilomyces lilacinus, a microbial fungus, has been explored for the first time as biological control agent against mosquito, *Culex quinquefasciatus* in order to curtail the excessive use of chemical insecticides. The fourth instar larvae of the mosquito were exposed to 1.0% mycelial suspension of *P. lilacinus*. It caused about 70% larval mortality and 16% adult emergence. The third instar larvae of *C. quinquefasciatus* exposed to different concentrations of Czapeckdox metabolites of the fungus. The ill effects evaluated on growth, development and mortality were non-significant ($P > .05$). Similarly, PYG metabolites of the fungus used against the third instar larvae of *C. quinquefasciatus* had a significant ($P < .01$) effect on the growth and mortality. The results indicate that the fungal mycelia can be a good bio-control agent against *Culex* mosquito. In Czapeckdox medium no toxic metabolite is produced, hence has no use in mosquito control but PYG metabolite appears to be a promising bio-control agent.

23-090

UTILISATION OF SPORE-PREPARATION AND METABOLITES OF A FUNGUS, *BEAUVERIA BASSIANA* IN BIOLOGICAL CONTROL OF *AEDES AEGYPTI*

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The first instar larvae of *Aedes aegypti* were exposed to spores of *Beauveria bassiana* suspended in distilled water at a concentration of 0.4×10^7 spores/ml. This resulted in 100% larval mortality in nine days. Escalated dose of 1.6×10^7 spores/ml, however proved fatal in seven days. The aged larvae appeared more susceptible. The later dose killed all the third instar larvae in three days.

The fungal metabolites in Gelatin medium caused a significant mortality at a high concentration (25%) and reduced the survival time from 9.85 days in control to 2 days. Strangely the low dosages (3%) and below stimulated the growth resulting in high growth index of the larvae. On the other hand metamobites in PYG medium did not induce any stimulating effects. Metabolites in Czapeckdox medium accelerated the egg laying rate which was most pronounced at 6.25% concentration. Increased concentrations however, showed a dose-dependent reversal of this effect.

The metabolites were found to be thermolabile.

23-091

EFFECTS OF ACETONE EXTRACT OF NEEM (*AZADIRACHTA INDICA* A. JUSS) SEED COAT AGAINST *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* TREATED AS THE FIRST INSTAR LARVAE.

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Acetone extract of neem (*Azadirachta indica* A. Juss) seed coat (ACNSC) was used against *Aedes aegypti* and *Culex quinquefasciatus* to assess its toxicity, growth regulating capacity and impact on hatchability. When *Ae. aegypti* Larvae in their first instar were exposed to a low concentration (10 ppm) of ACNSC, no mortality was observed. The larval mortality occurred with increased doses in a dose-dependent manner. A concentration of 40 ppm caused 100% mortality of the first instar larvae. While in *Cx. quinquefasciatus* mortality of same magnitude was caused by a dose of 20 ppm. Even at lower concentration of 10 ppm, 13.3% pupal mortality was observed in *Cx. quinquefasciatus* but no pupal casualty occurred in *Ae. aegypti*. Insect growth effect of ACNSC assessed in both the species but in *Ae. aegypti* the effect was more pronounced and a severe effect was seen at the eclosion. ACNSC (50ppm to 250 ppm) was administered on eggs of both the species but no adverse effect on hatching was seen.

23-092

SEQUENCE DIFFERENCES IN THE MITOCHONDRIAL CYTOCHROME OXIDASE I-II GENES OF SPECIES A AND B OF *ANOPHELES CULICIFACIES*

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Anopheles culicifacies is known to be a species complex comprising at least four species that include important malaria vectors in the Middle East and Indian sub-continent. Here we present evidence that two of the species, A and B, can be differentiated by their mitochondrial DNA haplotype.

DNA was prepared from individual field-collected specimens from Iran, Pakistan and Sri Lanka. Following polymerase chain reaction (PCR) amplification of a 1500bp segment of the cytochrome oxidase I and II genes, the amplification product was used for restriction mapping. Using seven restriction enzymes, 21 different restriction sites were found in the PCR product. Three enzymes, EcoRV, HinfI and AluI, gave a total of 14 sites of which 9 differed between two species.

A 766bp segment of the COI gene was sequenced. Nucleotide differences between the two species were observed at 46 positions, mostly T→C transitions (54.4%).

Although it remains to be confirmed that these mitochondrial haplotype differences are consistent throughout the species range, the evidence indicates substantial differences between COI-II genes of species A and B, at least as great as those seen between species of *Anopheles gambiae* complex (Oshaghi and Townson, unpublished observation).

23-093

COMBINED DEPRESSION OF FLIES QUANTITY AT FARMS AND URBAN OBJECTS USING CHEMOSTERILANTS, INSECTICIDES AND REGULATORS OF INSECTS DEVELOPMENT (RID)

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Methodics principles of sex chemical sterilization (food attractions with dimatif, thioTEF, hemf) were elaborated using natural flies populations. This method has high effectiveness (98,1-99,5%) and is the only way for the struggle with flies at the isolated territories: dry sugar food attractive at the aride zone and damp one at the temperate zone. Flies quantity reduction was registered in 2 weeks, clutch reduction (untill 41 eggs in a clutch) - in a month, the survival of progeny wasn't exceed 16% towards the end of 6th month (with corresponding control value 98%). The degree of nature flies populations sterility (at 10 large stock-breeding farms and 238 urban objects) wasn't exceed 41% owing to insufficient engage of attractions and flies getting from outside.

The employment of imagocides (carbophos, DDVF, ambush), larvicides (dimilin, alcistine, methopren) and adhesive glue compositions in addition to chemosterilants increased the effect till 99,2% during the periods of maximal flies quantity. Larvicides (JHA and CSI) ensured flies exclusion absence during 25-28 days. The combination "chemosterilant - feromone" (cis-9-trikozen, 1 mg per rubber dispenser, 10-20 traps per hectare) has high efficiency. Recommendations are developed for integral system of struggle with flies.

23-095

ERADICATION OF GLOSSINA AUSTENI ON UNGUJA ISLAND (ZANZIBAR) BY THE STERILE INSECT TECHNIQUE (SIT)

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The one tsetse species present on Unguja Island, *Glossina austeni* Newstead, is responsible for the cyclical transmission of African animal trypanosomosis. Efforts between 1986 and 1993 substantially suppressed the *G. austeni* population by applying pour-on insecticides to cattle and deploying insecticide-treated screens but this did not result in eradication.

In order to achieve eradication, the SIT was integrated into these efforts. At Tanga, a colony of 400,000 flies is being maintained. Leaving the sexes together after mating at a ratio of 1 male to 5 females, improved rearing efficiency. The colony currently provides 40,000 sterile males for release weekly. Gamma-sterilized flies, packed in special paper boxes, are released twice a week through a chute in an aircraft. This permits even dispersal of sterile males, also over areas that are inaccessible from the ground. Two million sterile males were released by air within 16 months.

As of May 1995, the numbers of sterile males released were sufficient to exceed the desired ratio of 15 sterile to 1 wild male in the field. Fly densities are monitored with sticky blue/white panels. The impact of sterile male releases, as revealed by fly population monitorings, are: (i) exponentially increased levels of induced sterility in wild females, as shown by dissection of trapped females, and, consequently, (ii) a progressive decline in the wild fly population density. A collapse of the target tsetse population is anticipated by 1997 at the latest. To detect very low levels of fly activity, blood samples from sentinel herd cattle are monitored for the transmission of trypanosomosis. Parasitological and immunological tests are used. With the former method, no transmission was detected in a large part of the island during recent surveys.

23-094

THE USE OF SANITATION TO REDUCE STABLE FLY (DIPTERA: MUSCIDAE) POPULATIONS IN BEEF CATTLE FEEDLOTS

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The stable fly causes \$400 million in losses to the United States cattle industry annually. Sanitation (waste management) has been long recommended as a means of reducing stable fly populations at cattle feedlots, but there is little published research to support the recommendation. Therefore, we conducted complete sanitation studies for two years in beef cattle feedlots in Nebraska. We found that this procedure reduced stable fly populations as much as 50%. In most cases this reduced the stable fly populations below the economic injury level.

23-096

DETECTION OF MOLECULAR VARIATION IN *AN. GAMBIAE* COMPLEX USING RAPD-PCR

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Random amplified polymorphic DNA (RAPD)-PCR is a powerful tool for fingerprinting anonymous genomes. We have analyzed genomic DNA and developed RAPD-based diagnostic molecular markers for *An. gambiae* complex species from different part of Africa. After studying 54 arbitrary primers, we found three primers one ten-mer and two 17-mer that permitted differentiation of all six members of *An. gambiae* complex: *An. gambiae* s.s., *An. arabiensis*, *An. merus*, *An. melas*, *An. quadriannulatus*, and *An. bwambae*. However some primers from the OPA series showed extensive intra-specific variation within species of *An. gambiae* complex.

In terms of reproducibility of RAPD products, our results demonstrate that this technique is sensitive to the temperature profile, DNA concentration and extraction method, and the source of Taq polymerase. We found that 5 pg of Phenol extracted DNA produce more and sharper bands than higher concentrations. Taq polymerase from some sources gave bands in the negative controls (all reagents except template DNA) presumably because of contaminating bacterial DNA. A two step program with an annealing temperature of 36 and 50°C proved most suitable.

23-097

Studies of inter- and intra-specific genetic variation in malaria vectors using PCR primers targeted at random sequences and simple sequence repeats

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We have used RAPD-PCR (random amplified polymorphic DNA polymerase chain reaction) to differentiate field-collected specimens of species A and B of the *An. culicifacies* complex from the Middle East and Indian subcontinent. One RAPD 10-mer primer produced two fragments, a 630 bp band specific to species B and a 420 bp band common to both species. A 17-mer primer also differentiated species A and B but showed additional variation in Iranian samples from different collection sites. 'Fingerprints' characteristic of the two species were obtained following PCR with a primer carrying a 3' anchored dinucleotide repeat.

In *An. stephensi*, strain and sample-specific RAPD patterns were obtained for seven laboratory strains and field samples originating from the Middle East and India.

Specimens of *An. subpictus* from inland and coastal sites in Sri Lanka showed two distinct fingerprints following PCR with a 3' anchored dinucleotide repeat primer.

This study demonstrates the value of RAPD primers and those targeted at simple sequence repeats, not only in differentiating seemingly isomorphic species but also in defining genetic differences between populations within a species, both factors of importance in malaria epidemiology.

23-099

BIOEFFICACY OF HEXANE EXTRACT OF KARANJA (*PONGAMIA GLABRA* VENT) SEED KERNEL- AGAINST MOSQUITO.

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Hexane extract of karanja, *Pongamia glabra* Vent (Fabaceae) seed kernel (HKSK) ranging at the concentrations of 4 ppm to 28 ppm was evaluated against the freshly emerged larvae of *Aedes aegypti* and *Culex quinquefasciatus*. Treatment of HKSK imparted the mortality that was correlated with the level of dosage. Before pupation, 100% fatality occurred in *Ae. aegypti* at a concentration of 20 ppm whereas in *Cx. quinquefasciatus* the same degree of mortality occurred at the extract concentration of 12 ppm in the first instar only. At low doses, the ensuing adults of *Cx. quinquefasciatus* had deformed legs, wings and truncated abdomen but the adults of *Ae. aegypti* showed no deformities. The developmental period was prolonged significantly in a dose-dependent manner for both the species treated. In *Cx. quinquefasciatus* this effect was more pronounced because of increased intermoult period showing an IGR effect of the extract. The calculated growth index also decreased in dose-dependent manner significantly more in *Cx. quinquefasciatus*. Indeed, *Ae. aegypti* appeared more vulnerable to HKSK than *Cx. quinquefasciatus*.

23-098

A DIAGNOSTIC TEST BASED ON SEQUENCE DIFFERENCES IN THE RIBOSOMAL DNA OF SPECIES A AND B OF THE *ANOPHELES CULICIFACIES* COMPLEX (DIPTERA: CULICIDAE).

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We have sequenced the second internal transcribed spacer (ITS2) of the ribosomal DNA (rDNA) of species A and B of the *Anopheles culicifacies* complex and identified a number of species-specific differences.

Based on these sequence differences, two primers were designed and tested in a Polymerase Chain Reaction (PCR) together with a conserved 5.8S primer and produced an amplification product of 250bp with target DNA from species A and one of 410bp with species B.

This PCR assay has given consistent and reproducible results with individual mosquitoes of field samples from Iran, Pakistan, India and Sri Lanka and correctly differentiates species A and B in the progeny of chromosomally-identified adults. Sequence data for the mitochondrial cytochrome oxidase I gene (Oshagi and Townson, unpublished observations) also readily differentiates these two species.

23-100

OVARIOLE BASAL BODY DEVELOPMENT AND PHYSIOLOGICAL AGE OF HAEMATOPHAGOUS DIPTERA

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Females of several dipteran families (Culicidae, Simuliidae, Tabanidae) possess in each ovariole a group of six to nine specialized epithelial cells in a region of the calyx wall that is enclosed by the end of the ovariole sheath is termed the basal body.

In all ovarioles of nulliparous females, and in those ovarioles of parous females in which ovulation had apparently not occurred, the cells of the basal body did not appear granular. However, after the first mature oocyte had entered the calyx lumen through the basal body, granulation occurred. Such a basal body is designated as a granular basal body. The size and intensity of granular basal bodies increased after every subsequent oviposition.

The granular basal bodies differ from all previous described ovarian structures. They may have a role in recycling used materials including egg sac remnants after every oviposition.

Examination of the granular basal bodies in intact ovaries, supravitality stained with neutral red, provided an easy method for distinguishing parous from nulliparous females, and has potential as a new method of age grading.

23-101

Study of Leptoconops in China
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Leptoconops are biting midges of Leptoconopinae, Ceratopogonidae. According to literature, Leptoconops in Neimenggu were reported by Okada early in 1954. Leptoconops (Holoconops) yunhsiensis in Hanshui River valley of Wudang Mountain, Hubei Province were collected by Yixin Yu in 1957. For the first time, a new species of Leptoconops was named by Chinese scholar in study history of Ceratopogonidae. New species of Leptoconops in Fujian, Yunnan and Tibet province were reported respectively by Keqin Sun and Tiesheng Li in 1968 and 1978. "Study of Leptoconops in Northwest China" were published by Zengjia Liu in 1990. For the first time, a monograph of Leptoconops were published by Chinese scholar in study history of Ceratopogonidae. Up to now, 34 species of Leptoconops respectively belonging to 3 Subleptoconops, were found: 28 species of Holoconops, 5 species of Leptoconops and 1 species of Acanthoconops. They are distributed in these areas: 15 species (44.12%) in Xingjiang, 2 species (5.88%) in Qinghai, 11 species (32.35%) in Ningxia, 8 species (23.53%) in Gansu, 2 species (5.88%) in Shanxi, 7 species (20.59%) in Neimenggu, 2 species (5.88%) in Tibet, 1 species (2.94%) in Sichuan, Hebei, Shandong, Hubei, Fujian, 3 species (8.82%) in Yunnan, and 2 species (5.88%) in Hainan province. Ecology and biology of common species in Leptoconops were investigated by author from 1986 to 1994. Suggestion of prevention and treatment of Leptoconops was also put forward by the author.

Key words Diptera Ceratopogonidae Leptoconops Holoconops Acanthoconops People's Republic of China.

23-103

NOTES ON THE GENUS MACRONYSSUS KOLENATI, 1858 FROM CHINA, WITH DESCRIPTIONS OF TWO NEW SPECIES (ACARI:MACRONYSSIDAE)
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This paper notes seventeen species of genus Macronyssus from China, among which Macronyssus emeicasis sp. nov. and M. fujianensis sp. nov. were two new species. The authors revised the generic characters of Macronyssus noted by Radovsky (1967), and erected three species-groups in this genus.

Macronyssus emeicasis sp. nov.

The new species closely resembles M. taiyuannensis Tian et Gu, 1992, but with the following differences: The new species with 15 pairs of dorsal-lateral setae, with 1 spine on the fixed digit of chela, with 7 setae on genital-ventral plate.

Macronyssus fujianensis sp. nov.

The new species is in the ventralis-group, and that can be readily distinguished from the other species of the species group.

Key words Acari Macronyssidae Macronyssus
Ichoronyssus species-group new species

23-102

INTRASPECIFIC VARIATION OF IXODES PERSULCATUS,
MAIN VECTOR OF THE LYME DISEASE AGENTS IN
EURASIA (IXODOIDEA: IXODIDAE)

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The multidimensional scaling method by means of software package SYSTAT has been used for estimation of relationships between eight geographical localities (populations) on the basis of morphometrical data. Eleven corresponding characters of males and females have been studied. The differences between the population are retracted only from absolute sizes of organs, whereas their proportions (i.e. shape) were constant in all geographical localities. The largest sizes are typical of populations from relict Tertiary landscapes of Primorski Territory and Altai with which areas of ecological optimum of Ixodes persulcatus coincide. The smallest sizes are observed in European populations near the north-western boundary of the distribution range of the species as well as in Alpine population of Tien-Shan Mountains, near the upper vertical boundary of the of the distribution range.

23-104

RECENT ADVANCES IN FORENSIC ENTOMOLOGY IN
ARGENTINA

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Arthropod succession in corpses at Buenos Aires (34° 36' S) differs somewhat from that accepted by European authors. Winter does not interrupt insect cycles indoors. Phaenicia sericata and Calliphora vicina are the most usual first wave species; the first is present from Spring to late Autumn. Sarcophagidae are not rare as first wave. Ants quickly attack small carcasses, rarely human corpses. Chrysomya albiceps appears in rural environment where it predominates, but also in urban locations. Both this species and C. megacephala have been found from the seventh day onward, and appear to prey on other species. Ophyra sp. appeared on exhumed corpses after four months; indoors after six months; also on exposed corpses. Adults appear to be active in the warm season. Fannia scalaris was often found with the former species, even in urban environment, although said to be a rural species. The phorid Megaselia scalaris was found indoors. Dermestes ater appears after 1 month, D. maculatus after 80-90 days. Necrobia ruficollis is frequent indoors after 1 month; N. rufipes was found in a single instance. Vespula germanica appeared in Patagonia (c. 39° S). Post-Mortem intervals are often shorter than those given by usual reference texts, and approach those determined by Goff and collaborators for Hawaii¹.

23-105

AMBLIOMMA TESTUDINARIUM KOCH (ACARI: IXODIDAE) INFESTATION IN GAYALS (*BOS FRONTALIS*) IN BANGLADESH

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Amblyomma testudinarium, the tortoise shaped tick is known to occur in a wide variety of hosts in the woodlands from Ceylon (Sri Lanka) and India to Borneo, Indonesia, Thailand, Vietnam, the Philippines, Taiwan, and southern islands of Japan. Recently, the distribution of this tick species has been reported from the north-eastern and south-eastern hills of Bangladesh. The hosts of adult *A. testudinarium* in these areas included pigs and cattle. This report deals with *A. testudinarium* infestation in gayals (*Bos frontalis*) from the hills of Naikhonchari, Bandarban, Bangladesh. The gayals are usually feral but domestication has been possible.

Tick collections from 15 captive gayals at 15 days interval between July 1994 and June 1995 revealed 282 adult *A. testudinarium*. Of them, 99 were female and 183 male with a ratio of 1:1.9. Maximum number of adult *A. testudinarium* on the host body was observed in the dry months (November to February) and minimum in the wet months (June to October). On an average an engorged *A. testudinarium* female weighed 4.5 gm against that of unengorged female (0.9 gm) from the same host. An adult female *A. testudinarium* sucked about 11 times blood than that of *Boophilus microplus*. *A. testudinarium* affected mostly the hidden parts of the body, specially the udder and/or scrotum, and the escutcheon. Because of long mouth parts this tick often inflicted wounds and invasion of maggots occurred.

23-107

MALARIA TRANSMISSION IN BINKO A SOUTH SUDAN SAVANNA VILLAGE OF MALI

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A study on malaria transmission by cross-sectional entomological and parasitological surveys was conducted from June 1994 to January 1995 in the village of Binko located in the South Sudan Savanna area of Mali, 2 km from the irrigated area of the Selingué dam.

The average parasite and gametocyte rates for the children of 2-9 years old were $57.7 \pm 3\%$ and $9 \pm 2\%$ respectively. The parasite rates showed significant seasonal variations ($P < 0.001$), with the minimum of $34 \pm 7\%$ in June and maxima of about $77 \pm 7\%$ in September and January.

An. gambiae s.s. was the main malaria vector found (99.5%, $n=621$). Its mean monthly man biting rate of 101.6 ± 1.2 bites/man/night (b/m/n) showed seasonal variations in relation to rainfall and irrigated rice cultivation pattern. The man biting rate was lower in June (2.38 b/m/n) and higher in August (253 b/m/n). The mean monthly entomological inoculation rate was 69 infective bites/man/month. This rate varied between 5.4 in June and 154 in August. The malaria transmission stability index for the study period was 3.1.

These results categorize Binko as a meso to hyperendemic area with stable malaria.

23-106

THE ROLE OF HOUSEFLIES (*MUSCA DOMESTICA*) IN TRANSMITTING THE MASTITIC AGENT *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* IN DAIRY HERDS IN ISRAEL

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Corynebacterium pseudotuberculosis is a facultative intracellular parasite, characterized by being pyogenic with suppuration and abscess formation in the lymphatic system in many domestic animals. Occasionally abscesses occur in visceral organs and the pathogen may also cause mastitis. Only rare cases of *C. pseudotuberculosis* mastitis in cattle have been recorded, however, our study was conducted in order to assess the role of houseflies in the transmission of *C. pseudotuberculosis* in dairy farms in Israel. The bacterium was isolated in June from 40 wild houseflies collected over a cow lesion and also from 28 laboratory flies fed on infected milk from a mastitic cow. In 10 out of 160 flies dipped entirely into a bacterial broth, the bacterium was recovered from the external body 10 min later. In 10 out of 40 flies fed on contaminated milk the bacterium was recovered from the external body 5 min later. When 110 flies were fed on contaminated sugar cubes, the bacterium was externally recovered from 70 of them 5 min later; from an additional 20 flies it was externally recovered 10 min post feeding. Eighty out of 110 flies excreted bacteria in their saliva from 5 min to 3 h post feeding on contaminated milk. In 40 out of 60 flies bacteria were isolated from their intestine between 1 and 4 h post feeding on contaminated milk. In feces of 30 out of 60 flies bacteria were found between 1 and 4 h post feeding on contaminated milk. In light of these findings and the fact that houseflies have a predilection to feed on milk residues on cow teats, we conclude that this insect plays an important role in the dissemination of *C. pseudotuberculosis* in dairy herds in Israel. In contrast stable flies (*Stomoxys calcitrans*) are not important in the transmission, since following membrane feeding on a mixture of bacterial broth and blood, the bacteria were not recovered after 5, 10, 15 and 30 min and 2 and 24 h post feeding.

23-108

BLOWFLIES (DIPTERA, CALLIPHORIDAE) CAUSED MYIASIS IN THE CENTRAL BLACK SOIL REGION OF RUSSIA

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I investigated cases of myiasis caused by blowflies in 1980-1995. Obligate myiasis caused by *Lucilia bufonivora* Moniez in amphibians (6 cases) and by *Protocalliphora azurea* Fallen (38 cases) as well as *Trypocalliphora braueri* Hendel (33 cases) in some birds. In the Region hosts for *L. bufonivora* were *Rana ridibunda* Pall., *R. esculenta* L., *R. temporaria* L. (fam. Ranidae). 8 species of birds from 5 families (Paridae-2, Muscicapidae-2, Turdidae-2, Sturnidae-1, Hirundinidae-1) are known as hosts for larvae of *P. azurea*. 14 species of birds from 8 families (Paridae-1, Alaudidae-1, Sylviidae-4, Motacillidae-2, Emberizidae-1, Ploceidae-2, Turdidae-2, Sturnidae-1) are hosts for larvae of *T. braueri*. Facultative myiasis caused in mammals (5 species) and man by *C. vicina* R.-D., *Lucilia illustris* Mg., *L. caesar* L., *Phormia regina* Mg. were studied. Hosts for *C. vicina* were *Erinaceus europaeus* L. (2 cases), *Sus scrofa* L. (1), sheeps (5) and man (3). Hosts for *L. illustris* were *Sciurus vulgaris* L. (1), *Sorex araneus* (1), *Alces alces* (1), sheeps (1), man (3). Two cases of myiasis caused in man by *Ph. regina* (1) and *L. caesar* (1) were studied.

23-109

SURVIVAL MODELS FOR *IXODES DAMMINI*E.P. Wileyto¹, S.G. Vail², and G. Smith¹.

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Models of *Borrelia burgdorferi* transmission dynamics must include submodels for survivorship of the *Ixodes* vector. In this paper we summarize the survivorship of *Ixodes dammini* under various combinations of temperature and humidity. We collected replicated cohort survival data for questing larvae, nymphs, and adults. Two kinds of fitting processes were compared: least squares regression and maximum-likelihood for conditional survivorship. Best fits were achieved using a separate model for each combination of temperature and humidity. However, it was possible to fit single models which included temperature and humidity without a serious gain in the sum of squared errors.

23-111

MAGGOT-HEAT VERSUS DAY-HEAT IN ESTIMATING POSTMORTEM INTERVAL

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In estimating the postmortem interval (PMI) and the age of maggots and puparia associated with a corpse, the method generally used is the summation of ambient heat units above a lower development threshold for the fly species involved and the comparison of the total units accumulated against known laboratory development rates. However, this method fails to take into account a number of variables that can influence that actual heat experienced by developing maggots.

One such variable, a major one, is the metabolic heat generated by massed maggots. Others include the rate of corpse cooling, solar radiant heat loading of the corpse and substrate and the influence of site topography, air movement, precipitation and overhead cover on microhabitats. Maggot heat dissipated into the corpse bed can influence the rate of development of the subcorpse community. Maggot heat also hinders the arresting of development while the corpse is cooled prior autopsy. However, the summation of heat units appears to work. It is perceived as an accurate, objective and proven method of analysis. The reasons for this will be discussed and areas needing further research identified.

23-110

BIOLOGICAL AND MORPHOLOGICAL ASPECTS OF *LONOMIA OBLIQUA* WALKER, 1855 (LEPIDOPTERA, SATURNIIDAE)L.M.Lorini¹ & E.Corseuil²

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Of the many Lepidopteran species distributed in south Brazil that may affect human beings with their urticant spines, there is one reported as being responsible for several deaths. These deaths were the result of haemorrhages caused by a toxin released from the caterpillar's spines onto the skin of the victim when touched. In order to determine some of the biological and morphological features of this previously unknown species, laboratory rearing was developed to measure the life span of the eggs, larvae, pupae and adults together with some of their physical features. The mean periods and sizes were as follows. The eggs were laid on the under surface of leaves and were ellipsoid in shape, 2.0 mm long, green in colour becoming darker nearer to hatching which occurred after 17.4 days. The caterpillars were dark brown, with longitudinal brown lines and many spines covering the whole body with colour changing from brown to black. They were gregarious, feeding mainly during the night on leaves of plants like *Platanus acerifolia* and some *Prunus* crop species. The duration of the caterpillar stage ranged from 63 to 76 days. The first larval instar was 5.6 mm long and the last 53.7 mm, and their head capsules were 1.0 mm and 5.0 mm wide respectively. The pupae were dark brown in colour with lengths of 30.2mm in females and 28.7 mm in males, and widths respectively of 10.9mm and 10.1 mm. The duration of the pupal stage ranged from 29 to 141 days. The male adult wings were 60.5 mm in length with yellow as the main colour, adult female wings with 80.2 long and mainly grey. The longevity of males was 5.9 days and females 7.7 days.

23-112

FACTORS AFFECTING THE PROBABILITY OF HOST AND TRAP LOCATION BY TSETSE FLIES (*GLOSSINA* SPP.) ON AN ISLAND IN ZIMBABWE

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Tsetse flies locate their hosts using visual and olfactory cues. Given that both sexes subsist exclusively on vertebrate blood, often in areas with low host density, they must be able to locate their hosts with high efficiency. Measuring this efficiency, and identifying the factors affecting it, is made difficult when the tsetse population density, and changes in it over time, are unknown. An experiment carried out on Antelope Island, Lake Kariba, provides a unique opportunity to produce efficiency estimates. A mark-recapture exercise was carried out over a period of more than four years, and weekly population estimates were obtained for males and females of *Glossina m. morsitans* Westwood and *G. pallidipes* Austen. The flies for marking were captured on ox fly-rounds and this provided an opportunity to estimate their probability of capture on a mobile host. It was found that male *G. m. morsitans* had a higher probability of capture than females of that species, and of both sexes of *G. pallidipes*. There was little difference between the sexes in the probabilities of capture in the latter species. These results can be explained on the basis of the reaction of tsetse to mobile baits in general, and to the presence of men in particular. The probability of capture by traps (either unbaited, or baited with various combinations and doses of acetone and carbon dioxide) showed precisely the opposite pattern; *G. pallidipes* was captured with a higher probability than *G. m. morsitans* and females with a higher probability than males. The effects of climatic variables, season and time of day are investigated.

23-113

CONTRIBUTIONS TO THE KNOWLEDGE OF THE LIFE-HISTORY OF THE *PHARYNGOMYIA PICTA* MEIGEN (DIPTERA, OESTRIDAE), AN ENDOPARASITE OF THE *CERVUS ELAPHUS* LINNAEUS (ARTIODACTYLA, CERVIDAE).

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Actually, in different zones of Spain, *Pharyngomyia picta* Meigen is relatively abundant and causes sanitary disorders to the *Cervus elaphus* L. populations. For this reason the study of this bot fly can be considered a very important research also under a practical point of view.

Ph. picta lives as endoparasite reaching the larval maturity in the retropharyngeal cavity of the host.

This work tries to give a contribution to the bionomical knowledge of *Ph. picta*, starting from observations achieved in Monte de Lugar Nuevo (Jaén, Spain).

23-115

PICTORIAL KEY TO MAIN SPECIES OF SIPHONAPTERA OF MEDICAL AND VETERINARY IMPORTANCE IN BRAZIL
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With the objective of creating a pictorial key that makes an easy identification of the main species of fleas of medical and veterinary interest, there have been examined, in the course of the year of 1995, specimens of several brazilians institutions.

The species used for the construction of the key were: *Tunga penetrans* (Linnaeus, 1758), *Echidnophaga gallinacea* (Westwood, 1875), *Ctenocephalides felis* (Bouché, 1835), *Ctenocephalides canis* (Curtis, 1826), *Pulex irritans* Linnaeus, 1758, *Xenopsylla cheopis* (Rothschild, 1903), *Xenopsylla brasiliensis* (Baker, 1904), *Leptopsylla segnis* (Schonherr, 1816), *Nosopsyllus fasciatus* (Bosc, 1801) and *Polygenis* spp.

There have been considered, mainly, the following characteristics: eyes, format of the head, occiput seta, genal and pronotal ctenidium, hind coxa, hind tibia, segments thoracic, antesensilial seta and spermatheca.

23-114

ODOUR-GUIDED HOST-FINDING BY HAEMATOPHAGOUS MOSQUITOES: THE EFFECT OF HOST-SPECIFIC ODOURS ON THE BEHAVIOUR OF MALARIA MOSQUITOES (DIPTERA; CULICIDAE).

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Female mosquitoes require a blood meal for egg production, and a variety of stimuli are involved in the vital process of location of a suitable host animal, including physical, visual and olfactory. The importance of olfactory stimuli has long been recognized, however, apart from carbon dioxide and lactic acid, no specific compounds have been indentified. By means of two-choice bioassay tests the effect of host-specific odours on the behaviour of members of the *Anopheles gambiae* complex, all acting as vectors of malaria in Africa, are studied. The responses of *Anopheles gambiae* s.s, the most antropophilic member of the complex, to (components of) human emanates are presented.

23-116

IMMUNE RESISTANCE TO TICKS
—STUDIES ON RED CELL IMMUNE FUNCTION OF HOST

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Since the phenomenon of acquired immune resistance to ticks following infestation was reported by Trager in the late 1930's, the host humoral and cellular immune resistance have been studied by many workers (wikel 1982, 1984, 1994, willadsen 1980, Brow, 1988; Opdebeeck 1994). Yet, it isn't clear what the red cell immune function of the host play in immunity to ticks. This article reports that the changes in RBC immune function of rabbits after re-infestation by *Ixodes sinensis* (Infestation group) and immunization using the SGA of *Ixodes sinensis* (immunization group). The RBC immune function includes C₃b receptor rosette rate (C₃bR)(%), immune complex rosette rate (ICR)(%) and phagocytosis of leukocytes promoted by RBC (%).

The result showed that in control group C₃bR was 6.37 ± 2.51(%), ICR was 8.67 ± 4.62(%), and phagocytosis of leukocytes promoted by RBC was 28.04 ± 6.24(%). The RBC immune function in both groups of infestation and immunization increased dramatically; and phagocytosis of their leukocytes promoted by RBC were 41.86 ± 10.29(%) and 54.21 ± 12.37(%) respectively, being significantly higher than that in control group (P < 0.01). ICR decreased and C₃bR increased as compared with those of the control group (P < 0.05). The paper suggested that the RBC immune function of host play an important part in immunity to ticks, besides antibody, complement, hypersensitivity leukocyte etc..

23-117

HISTOCHEMISTRY AND HISTOPATHOLOGY OF TICKS (*IXODES SINENSIS*) AFTER INFESTING IMMUNIZATION RABBITS.

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The ticks (*Ixodes sinensis*) fed on immunization rabbits with SGA from the adult female ticks were studied after 1, 2, 3, 5, 8 and 15 days of infestation respectively by using histochemical and histopathological techniques, as well as micrography and imaging analysis.

The histochemical results showed that the reactions of glycogen, protein, nucleic acid (RNA and DNA), AKP, ACP etc. in the immunization group were less than those of the control group ($P < 0.01$). The gray level assessment in tissue imaging showed significant difference between the two groups ($P < 0.05$). The histopathological examination of the *Ixodes sinensis* fed on immunization rabbits showed that the gut was damaged within 8 days. Significant difference was found in gut damages between the two groups. ($P < 0.001$). It is suggested that *Ixodes sinensis* infecting immunization rabbits had significant change on the above histochemical element and histopathological structures, which might be considered as important characteristics in immune to ticks.

23-119

LIFE CYCLE OF *DRYOMYZA FORMOSA* (WIEDEMANN) IN NAGASAKI, WESTERN JAPAN (DIPTERA: DRYOMYZIDAE)

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An investigation to know the life history of *Dryomyza formosa* (Wiedemann) was made in Nagasaki, Western Japan from January 1990 to December 1994. A trap baited with horse meat was set at three different altitudes, 5 m, 500 m and 1,000 m, of Mt. Gokahara-dake (alt. 1,058 m). The number of flies captured was counted once a month. Male and female flies appeared in spring (April to May) and autumn (November) at altitude 5 m. The two peaks of fluctuation were also found from April to June and October to November at altitude 500 m. However, no adult was trapped by the lowland traps during summer. One big peak of fluctuation was found in June, and some adult flies were trapped during summer at alt. 1,000 m. Wear of wing suggested the adults trapped at alt. 5 m and 500 m in spring were juvenile and teneral in contrast with old aged ones trapped in autumn. Percent wear of wing increased towards autumn at alt. 1,000 m. Percent females with inseminated spermathecae and % mature ovaries suggested that female flies were not sexually matured before October. Flies collected at alt. 5 m and 500 m in autumn and early winter all were already matured. Immature stages were not found in the trap baited with horse meat, but larvae were reared from human feces at alt. 50 m and 500 m during late autumn. Adults emerged from overwintered pupae on April and May. Juvenile adults emerged in lowland most probably migrate to highlands for overwintering. After aestivation adults matured and migrated to lowlands for laying eggs.

The result of the present field experiment showed that *D. formosa* is an univoltine, short-day insect. Diapausing adult most probably overwinters at the highland more than 1,000 m. After larval growth in late autumn, diapausing pupa overwinters for about 4 months.

23-118

FLIGHT ACTIVITY OF *PHLEBOTOMUS ALEXANDRI* USING A VEHICLE-MOUNTED NET IN THE SULTANATE OF OMAN.

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A vehicle-mounted net was used to collect hourly samples of sandflies on 15 nights during June in northern Oman. *Phlebotomus alexandri* Sinton was the most abundant of the 7 sandfly species collected.

63 % of the *P. alexandri* were female and most were unfed, with only 8 % of the females being blood-fed and 18 % gravid. Of the unfed females, only 9 % were parous.

Activity was almost entirely nocturnal. Apart from the light intensity, a multiple regression showed that the most important factor encouraging flight was low humidity, followed by low wind velocity, and to a small extent, high nighttime temperatures.

During the night, activity was greatest during light intensities < 0.1 lux, although a few males and unfed females were collected up to 1.25 hours after sunrise ($< 50,000$ lux). Both male and female *P. alexandri* showed two peaks of activity during the night, with reduced catches around 01.00h. An exception was the blood fed females, which were largely restricted to the first half of the night (20.00 - 01.00h). Parous females were more abundant during the second half of the night (unlike the nulliparous flies), but the numbers of parous flies collected was too small to be statistically analysed.

23-120

CHARACTERIZATION OF NITRIC OXIDE SYNTHASE EXPRESSION DURING MALARIA PARASITE DEVELOPMENT IN *ANOPHELES STEPHENSI* (LISTON) (DIPTERA: CULICIDAE)

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In both vertebrate and invertebrate hosts, malaria parasites overcome diverse mechanical and physiological barriers to invasion and multiplication. We know little, however, about interactions between the mosquito and *Plasmodium* and strategies the parasite may use to overcome or evade invertebrate host obstacles.

In vertebrates, nitric oxide synthase (NOS) catalyzes the production of nitric oxide which participates in signal transduction, pathogen destruction, and immune modulation. Using degenerate polymerase chain reaction (PCR) primers to *Drosophila* NOS (DNOS; Regulski and Tully, 1995. Proc. Natl. Acad. Sci. USA 92:9072-9076), we examined NOS expression in *Plasmodium falciparum*-infected *Anopheles stephensi*. High levels of NOS expression were detected at various stages of parasite development.

Sequence analysis of the NOS PCR-amplified from infected mosquitoes indicates a high level of sequence and amino acid homology to DNOS, suggesting that the fragment is derived from the *A. stephensi* genome. Parasite loss during sporogony even in susceptible *Anopheles* implies that inherent refractory mechanisms exist (Rosenberg and Rungsiwongse, 1991. Am. J. Trop. Med. Hyg. 45:574-577). Mosquito anti-*Plasmodium* NO could limit parasite development in a manner similar to vertebrate liver NO (Mellouk et al. 1991. J. Immunol. 146:3971-3976). We are continuing to characterize NOS gene expression in infected mosquitoes and the impact this may have on the success of parasite development.

23-121

USING SATELLITE IMAGERY TO PREDICT THE DISTRIBUTION OF AFRICAN HORSE SICKNESS AND *CULICOIDES* (DIPTERA: CERATOPOGONIDAE) VECTORS IN MOROCCO.

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Since 1993, 20 sites in Morocco have been monitored for the presence and abundance of *Culicoides imicola* (Diptera, Ceratopogonidae), a biting midge which was the vector of a recent epidemic of African horse sickness (AHS), a highly pathogenic viral disease of equines. The normalised difference vegetation index (NDVI), a remotely-sensed measure of green vegetation biomass, has proven to be a better explainer of the distribution of *C. imicola* than any of several ground-measures of climate, including air and soil temperature, humidity, saturation deficit and windspeed. Using discriminant analysis, the mean annual minimum NDVI (NDVI_{min}) from 1980-1991 correctly predicted the approximate abundance of *C. imicola* at 15 of the 20 sites in Morocco. Furthermore, the Moroccan relationship between NDVI_{min} and abundance successfully predicted the distribution of the vector in Spain. In general, NDVI_{min} is significantly correlated with rainfall and soil moisture and the relationship with the distribution of *C. imicola* may operate via the frequency of breeding sites (wet soil) of the insect. In Larache province of Morocco NDVI_{min} also appears to describe the distribution of AHS outbreaks during the epidemic.

23-123

EFFECTS OF *SIMULIUM DAMNOSUM* S. L. HAEMOLYMPH ON THE SURVIVAL OF *ONCHOCERCA VOLVULUS* THIRD STAGE LARVAE *IN VITRO* AND COMPARISON TO THE POSSIBLE SITUATION *IN VIVO*.

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Motility assays *in vitro* using immune *Simulium damnosum* s.l. haemolymph (infected with: *Escherichia coli* D.31, *Onchocerca ochengi* microfilariae or Lipopolysaccharide) demonstrated that it was more efficient at killing third stage *Onchocerca volvulus* larvae, than control and SHAM (medium injected) haemolymph. The same effect was observed when the haemolymph was boiled, suggesting that heat stable molecules are implicated in the killing mechanism. When the parasites were exposed to haemolymph *in vivo*, by intrathoracic injection, the situation observed was quite different, killing times were far shorter, implying that *in vivo* many other active components were present. Despite this, using transmission electron microscopy, very little ultra structural damage was observed in the parasite. The possible immune mechanisms used by *S. damnosum* s.l. against *O. volvulus* will be discussed.

23-122

THE EFFECT OF RODENT AND HUMAN MALARIA PARASITES UPON THE BLOOD-FEEDING AND REPRODUCTION OF ANOPHELINE MOSQUITOES

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The blood-meal size taken by anopheline mosquitoes of the same wing length and age has been determined by haemoglobinometry and by measurement of haematin excretion. *Anopheles stephensi*, feeding upon mice infected with *Plasmodium yoelii nigeriensis* take significantly larger blood meals when infected when the infective, gametocyte stage of the blood meal is present. Despite this increase in blood meal size, mosquitoes that become infected with malaria oocysts exhibit a 21-28% reduction in the number of eggs produced during the subsequent gonotrophic cycle. This fecundity reduction occurs at both high and low intensities of infection (<75 oocysts per midgut: 4.4 oocysts per midgut). Examination of the primary ovarian follicles at various intervals post-infected blood meal revealed that infection does not affect the initial development but that a significant number of follicles began to resorb after 24h. Vitellogenin uptake, monitored by grouping according to Christophers' stages is significantly retarded in the remaining follicles. These results are being confirmed using an ELISA.

Field studies conducted in Muheza, NE Tanzania, have demonstrated that human malaria parasites also significantly reduce the egg production of *Anopheles gambiae* s.l. This pathology may therefore have implications for our current understanding of vector population biology and disease transmission.

23-124

MORPHOLOGICAL, ETHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF LARVAE OF *CEPHENEMYIA AURIBARBIS* (MEIGEN) AND *PHARYNGOMYIA PICTA* (MEIGEN)

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Cephenemyia auribarbis (Meigen) and *Pharyngomyia picta* (Meigen) (Diptera Oestridae) are two important parasites of the respiratory tracts of the red deer (*Cervus elaphus* L.). Larval identification is usually made by examining external features pertaining to first or third instar larvae.

Morphological examination of second instar larvae showed discriminant characters. In particular the distance between the bases of the fleshy cones of the pseudocephalon is reduced in *C. auribarbis* and wide in *P. picta*; spines are present on the anterior part of the seventh abdominal segment in the former species and lack on the middle dorsal surface of it in the latter.

Moreover, a remarkable difference in the ethology of the first instar larvae was observed: *P. picta* larvae reach the trachea and the larger bronchi and then migrate into the nasal cavities little before the first molt, while *C. auribarbis* larvae always remain in the nasal cavities.

Also the electrophoretic analysis of the tetrazolium oxidase or superoxide dismutase (TO or SOD) enzyme system proved to be a reliable tool for the identification of the two species at the larval stage.

23-125

THE USE OF IMAGE ANALYSIS SYSTEMS IN SIMULIID TAXONOMY

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Medical and Veterinary Division, Department of Entomology, The Natural History Museum, London, U.K. - 1 Departamento de Entomologia, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil Recent work on Neotropical Simuliid vectors of human onchocerciasis, has shown that behavioural and vectorial differences can be attributed to the existence of sympatric populations of closely related species or of species complexes defined by cytological and molecular methods. A search for fine morphological characters for rapid field identification of members of species groups and complexes involves the need for simultaneous comparisons.

The "Synoptics" system of automated imaging of both morphological and chromosomal preparations allows a composite computer image to be constructed from various focal plains of specimens viewed through a microscope. This has surmounted previous problems of microphotographic reproductions that have precluded the full definition of morphological features in a single photograph because of depth of field problems.

This technique has been effectively used in differentiating *Simulium oyapockense* Floch & Abonnenc from *S. roraimense* Nunes de Mello, both vectors of onchocerciasis in the Brazilian Amazon, and from the morphologically similar non vector *S. minusculum* Lutz.

23-127

THE EXPRESSION OF PARASITE ANTIGENS IN THE SALIVARY GLANDS OF THE MOSQUITO, *Aedes aegypti*: A MODEL SYSTEM TO EXAMINE THE POSSIBILITY OF USING HAEMATOPHAGOUS INSECTS TO DELIVER VACCINE ANTIGENSS.L. Stowell, M.A. Hughes, R.E. Sinden¹, J.M. Crampton

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A model system has been chosen to explore the concept of vaccine delivery by haematophagous insects. The vaccine:insect combination selected to model and assess this novel possibility is:

- the transmission blocking antigen, Pbs21 which is expressed on the surface of the rodent malaria parasite *Plasmodium berghei* during zygote and ookinete stages of development and
- the mosquito *Aedes aegypti*.

The eventual aim is to produce a transgenic mosquito capable of expressing significant quantities of the Pbs21 antigen in its salivary gland such that a small amount of the antigen protein is delivered to the host, in the saliva, when the female mosquito takes a blood meal. To do this, the cloned gene coding for the Pbs21 antigen, under the control of a salivary gland specific promoter, will eventually be introduced into the mosquito genome using transgenic means.

Recombinant Pbs21 produced by *Spodoptera frugiperda* cells using a baculovirus expression system is highly immunogenic and has previously been shown to induce transmission-blocking activity in mice. Our initial studies have focused on expressing the gene coding for Pbs21 in cultured mosquito cells and salivary glands *in vitro*. The gene transfected into *Ae. aegypti* cultured cells is expressed and the recombinant Pbs21 protein is detectable immunologically. When the Pbs21 gene construct is transfected into cultured *Ae. aegypti* salivary glands, transcripts of the gene are detectable by *in situ* hybridisation, and Pbs21 antigen is also produced. Future studies will determine whether the Pbs21 antigen produced in *Ae. aegypti* salivary glands can induce transmission-blocking activity when introduced into mice.

23-126

SPATIAL AND TEMPORAL ANALYSIS OF MALARIA TRANSMISSION PATTERNS USING A GEOGRAPHIC INFORMATION SYSTEM

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Field studies were conducted in 15 villages bordering Lake Victoria in western Kenya, an area of intense malaria transmission. *Anopheles gambiae* complex and *An. funestus* Giles mosquitoes were sampled from over 800 households weekly over a two year period using a simple mosquito trap. Samples of *An. gambiae* complex specimens were identified to species as *An. gambiae* Giles or *An. arabiensis* Patton using PCR. Infection rates with *Plasmodium falciparum* were determined using ELISA for a sample of each of the three malaria vectors.

The Global Positioning System (GPS) was used to map all houses from which adult mosquitoes were sampled, rivers, the lake shore, possible larval habitats, swampy areas, and other physical features. Measurements of latitude and longitude were accurate to within 2 meters; altitude measurements were less accurate. GPS coordinates for each physical feature were entered into the ATLAS-GIS Geographic Information System software package. Analysis of abundance and entomologic inoculation rate for each of the three species in relation to distance from physical features at different seasons showed marked small-scale variability.

23-128

HOST-FINDING OF THE BLOODSUCKING BUG *Triatoma infestans* (HEMIPTERA: REDUVIIDAE), A VECTOR OF CHAGAS' DISEASE: AN OLFACTOMETER STUDY.

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The reactions of groups of 50 individuals of second instar *Triatoma infestans* (KLUG) to different sensory stimuli were examined using a T-shaped olfactometer. The air-speed in the olfactometer was approximately 2 m/s. The insects' behavior was measured as: (a) the percentage of insects entering the olfactometer during the course of a 30 minute experiment ('active insects'), and (b) the percentage of these active insects that entered the leg of the olfactometer where a stimulus was presented ('attracted insects'). Without a stimulus, circa 30% of the bugs were activated, and in each leg about 50% of these active insects were found. Laboratory mice or a human hand in the olfactometer activated more than 80% of the insects, and more than 95% of these were attracted.

Other stimulus sources were mostly taken from potential hosts of *Triatoma infestans*. Most effective was a combination of a previously worn cotton sock and CO₂ (concentration in the olfactometer between 0.05% and 0.1%). This combination activated more than 70% of the insects. Ca. 90% of these were attracted. A worn sock alone activated almost 60% of the bugs, and ca. 90% of them were attracted as well. These values were also obtained using a cotton cloth impregnated with the ethanol extract of a worn sock. Less effective were, among others, between 0.05% and 0.1% CO₂ alone (less than 60% activated / ca. 65% of these attracted), a mouse-nest (ca. 45% / ca. 90%), a skin extract obtained from M. GEIER (University of Regensburg) that is highly effective in the mosquito *Aedes aegypti* (ca. 35% / ca. 75%), and dried human saliva (ca. 35% / ca. 60%). Not effective was, among others, dried human urine. None of these stimuli was found to be as effective as a natural host.

A cotton cloth impregnated with 2 gr. of lactic acid alone did not seem to have any effect on the bugs' behavior. A combination with CO₂ (between 0.05% and 0.1%) activated as many insects as CO₂ alone. However, it was impossible to observe a significant attraction towards these combined stimuli. This was the only instance when an activation without attraction was observed.

23-129

THE ROLE OF CARBON DIOXIDE IN HOST FINDING BEHAVIOUR OF THE YELLOW FEVER MOSQUITO *Aedes Aegypti* (DIPTERA: CULICIDAE): A WIND TUNNEL STUDY

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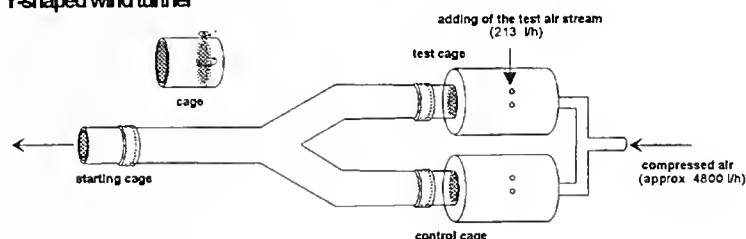


The effect of carbon dioxide stimuli on the behavioural response of female *Aedes aegypti* (L.) was tested in a Y-shaped wind tunnel as described by GEIER (PhD thesis, Regensburg 1995). CO₂ was presented alone in different concentrations, either continuously or pulsed, and in combination with lactic acid and 1-octen-3-ol.

Carbon dioxide of 8 different concentrations (0.001 - 6 vol-% above ambient air and 100 %) was added to a test air stream. Mosquitoes started to respond to carbon dioxide within the range of 0.01 to 0.2 vol-%. Carbon dioxide elicited significant activation (mosquitoes having left the starting cage) as well as attraction (mosquitoes in the test cage). More than 0.2 % carbon dioxide in the test air stream did not increase the number of responding mosquitoes. At the saturation level an average of more than 60 % of the test animals flew upwind and about 40 % arrived in the test cage. No response differences were observed when the CO₂ stimulus was presented either continuously or in intermittent pulses (pulse durations 13 - 0.13 s). CO₂ acts as a synergist for lactic acid (SMITH et al. (1970) *Ann. entomol. Soc. Am.* 63: 760-770), but when the background CO₂ concentration was reduced, lactic acid turned out to be attractive even in the absence of carbon dioxide. No synergism of CO₂ could be found with octenol, which was neither attractive singly nor did it increase the effect of carbon dioxide.

These results support the assumption that CO₂ alone can act as an activator and attractance. In the Y-shaped olfactometer it is not required to pulse the CO₂ stimulus in order to enhance the response. In contrast to previous studies (ACREE et al. (1968) *Science* 161: 1346-1347) it could be demonstrated that inspite its synergistic impact, carbon dioxide was not essential for the attractive effect of lactic acid.

Y-shaped wind tunnel



23-131

NOTES ON THE INCIDENCE OF THE *LUCILIA* GENUS (DIPTERA: CALLIPHORIDAE) IN UMBRIA, CENTRAL ITALY. A CASE OF MYIASIS BY *LUCILIA AMPULLACEA* (VILLEN 1922) IN *TESTUDO GRAECA*.

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During the period 1990-1995, we carried out some surveys on the incidence of the *Lucilia* genus in Umbria, enabling us to identify the main species present in our region: *L. sericata* (45%), *L. Richardsi* (22%), *L. illustris* (16%), *L. caesar* (9%) and *L. ampullacea* (2%), (other species 6%). These insects can cause facultative myiasis, generally following an altered physiological state of the host animal, for instance in case of diarrhea, nasal or vaginal leakage and above all of traumas. In the last few years in Umbria, various and recurrent cases of myiasis were recorded by us: dermal myiasis in sheep by *L. sericata*; dermal myiasis in dogs by *L. illustris* and *L. sericata*; aural myiasis in a rabbit by *L. caesar*; rectal myiasis in a cat by *L. sericata*.

A particularly interesting myiasis by *L. ampullacea*, a quite rare species in Umbria, was observed in a terrestrial turtle (*Testudo graeca*), which had been bitten by a dog in the month of August. *L. ampullacea* had effected three cycles of ovoposition in the animal's healthy skin plicae, under its shell and on its limbs, near the traumatic wound. A total number of 480 larvae at the first, second and third instar was counted. The larval development from the first to the third instar was obtained in laboratory at 29°C and 80 RH in just 3 days. Our successful therapy required the removal of all larvae from the lesion, which was kept open and was inspected for three days. As far as we know, that is the first case of myiasis by *L. ampullacea* recorded in Italy.

23-130

ISOLATION OF PATHOGENIC BACTERIA FROM *ALPHITOBIVS DIAPERINUS* (PANZER) (COLEOPTERA: TENEBRIONIDAE), A PEST OF POULTRY HOUSES.

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Alphitobius diaperinus is a darkling beetle, recently recorded by us in Italy for the damages caused by its larvae to poultry house insulation. Moreover, the larvae can harbour various pathogens of poultry and since they are found in a very great number in litters, that is a serious hygienical problem above all in broiler houses. In order to verify the pathogenic role of these beetles, we have effected four samplings (at about 15 days' intervals) of larvae and adults in an Umbrian broiler house massively infested. The insects were dipped in 1% formaldehyde for 1 minute and were dissected, in sterility conditions, to extract their content. This was cultured in Trypticase Soy Broth (BBL) at 37°C for 12 hours. Afterwards, cultures in Trypticase Soy Agar and Trypticase Soy Agar with addition of sheep defibrinated blood (5%) and Mac Conkey Agar (BBL) were made. The bacteria so developed were identified by biochemical and serological tests. *Enterobacter cloacae* was always isolated from larvae, whereas from adults *Streptococcus dysgalactiae* was isolated twice, *Staphylococcus intermedius* was isolated once and once the sampling turned out to be sterile. *E. cloacae* is a microorganism frequently isolated either from human or avian intestine, but it is not considered to be enteropathogenic. On the contrary, *S. dysgalactiae* and *S. intermedius* can cause pneumonia, conjunctivitis, otitis and urogenital infections to poultry.

23-132

MALLOPHAGA MENOPONIDAE: NEW PARATENIC HOSTS OF *SYNGAMUS TRACHEA* (NEMATODA: SYNGAMIDAE) IN *ALECTORIS RUFA*

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It is known that *Syngamus trachea*, a parasite frequently occurring in the respiratory apparatus of fowls, can have a direct or an indirect life cycle. That is important from an epidemiological point of view because the intermediate host can harbour parasites for long. The usual intermediate hosts of *S. trachea* are earthworms (*Annelida*), which swallow the parasite's larvae by feeding on infected faeces on the ground. We could demonstrate that also biting lice (*Mallophaga*) can be paratenic hosts of *S. trachea*, like earthworms. Our observations started with a case of a red-legged partridge (*Alectoris rufa*) infested by *S. trachea*, also showing a severe infestation by biting lice. At a microscope examination, five of those insects also showed the presence of nematode larvae, presumably *S. trachea*. To reach an exact identification, we carried out an experiment consisting in making a healthy sample of partridge swallow four biting lice containing nematode larvae, still alive. The larval migration of the nematodes could not be observed, for the experiment was effected on just one bird, but two weeks after the laboratory contamination, at least two specimens of *S. trachea* adults were observed in the bird's trachea; after the 22nd day eggs of the nematode were present in the bird's faeces. If we consider that *Mallophaga* are obligatory parasites of fowls and they do not feed on earth contaminated by faeces, as earthworms do, it can be assumed that their infestation can occur when the host's feathers and skin are dirty of faeces containing a high number of *S. trachea* eggs.

23-133

HUMAN SKIN MYIASIS BY *GASTEROPHILUS INTESTINALIS* (DE GEER) (DIPTERA: GASTEROPHILIDAE) IN UMBRIA, CENTRAL ITALYM. Principato, J. Azzopardi, V. Cuteri¹

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The oral and gastric myiasis in horses by *Gasterophilus intestinalis* is, in Umbria, the most frequent parasitosis of those caused by the other four *Gasterophilus* species we have recorded in this region. The infestation occurs in the 96% of half-free-ranging horses, with a parasitic charging potential that, already in February, reaches N.450 larvae in the host's stomach. Reported herein for the first time in Umbria are three cases of human skin myiasis caused by *G. intestinalis* first instar larvae, recorded in 1994 and in 1995, from the end of October to mid-November, in people who had ridden horses still infested by *Gasterophilus* eggs. The initial stage of the parasitosis was characterized by the formation of a very itching tiny blister leading to scratching. Then a well visible small channel formed, followed by painful swelling and a suppurative process by *Streptococcus pyogenes*. The result was an open lesion, out of which blood serum and pus leaked. The most affected areas (with 7/10 larval attacks) were the thighs, the calves and the pelvis. Lesions on the arms were rare. The recovery was obtained after squeezing lesions up to the complete removal of parasites (dead) and after giving Tetracycline. We think that, from an epidemiological point of view, the period October/November is a particularly dangerous moment, since those *Gasterophilus* larvae that could not reach the host are led to do an extreme attempt of adapting themselves by penetrating any host within their range.

23-135

NEW ECOLOGICAL FEATURES OF *Aedes aegypti* (L.) (DIPTERA: CULICIDAE) AND THEIR CONSEQUENCES ON DENGUE TRANSMISSION CYCLESF. Fouque, J. M. Reynes, R. Carinci, P. Gaborit, E. Telliam¹ and J. Fargette¹Laboratoire d'Entomologie médicale, Institut Pasteur de Guyane, Cayenne, French Guiana - ¹ Laboratoire de Virologie, Institut Pasteur de Guyane, Cayenne, French Guiana

The mosquito *Aedes aegypti* is the only vector of dengue in French Guiana where this disease is endemic since many years. The ecological preferences of *Ae. aegypti* were supposed to be well known, and out of Africa this species was generally recognized as urban, almost exclusively anthropophilic and as a good vector but not a reservoir for dengue viruses.

However, during a two-years survey of the dengue situation through entomological data in French Guiana, *Ae. aegypti* was found not only into urban environment, but also into rural and wild environment, even breeding into the rain forest. The anthropophilic rates were found to be less than 0.5, implying that *Ae. aegypti* commonly feed on non-human hosts. Furthermore, a vertical transmission rate of dengue viruses of 1 infected mosquito per about 2000 tested mosquitoes was found, suggesting that *Ae. aegypti* can also behave as a reservoir for dengue. These findings arise the question of the existence of a selvatic cycle of dengue into the rain forest of French Guiana. Though, a selvatic cycle of dengue in America has not been demonstrated, the ecology of *Ae. aegypti* is not a limiting factor.

23-134

FORENSIC ENTOMOLOGY IN SWITZERLAND.

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Forensic entomology was initiated in the Canton de Vaud (Switzerland) in 1993. Since that time, 31 cases have been analysed. About 15 species of flies (Diptera), mainly belonging to the genera *Calliphora*, *Lucilia*, *Sarcophaga*, *Fannia*, *Piophilina*, *Megaselia* and *Hydrotaea* were collected. Different samples of these species were reared in order to determine the postmortem interval (PMI). Using data from the literature as well as laboratory data, PMI was successfully determined in most cases. The discovery of *Chrysomya albiceps* (Wiedemann) (Calliphoridae) was rather surprising. Moreover, *Syrphid pipiens* (L.) (Syrphidae) found on carrion in two cases, has not been considered until now as a forensic species. Detailed analysis of two cases will be presented and discussed.

23-136

A GENETIC ANALYSIS OF THE MELANOTIC ENCAPSULATION PHENOTYPE OF A *PLASMODIUM*-REFRACTORY STRAIN OF *ANOPHELES GAMBIAE*

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A genetically selected *Plasmodium*-refractory strain of *Anopheles gambiae* melanotically encapsulates many species of *Plasmodium*. Genetic studies of this refractory strain and a selected susceptible strain have shown that the refractory phenotype is controlled by a limited number of genes. A model system that uses CM-Sephadex beads instead of the malaria parasite has the potential to simplify studies of the genetic mechanism of this immune response. We have recently shown that the bead melanization phenotype has a similar pattern of inheritance to the *P. cynomolgi* B encapsulation phenotype, and, in collaboration with A. Cornel and F. Collins (CDC, Atlanta, GA), that these two encapsulation phenotypes have a common genetic mechanism. Genetic mapping experiments suggest that a major gene involved in the melanization response is located on the right arm of the second chromosome. Results of genetic mapping using RFLP and microsatellite markers from 2R will be reported. In addition, results of an analysis of encapsulation responses to beads with various characteristics will be discussed in regard to possible molecular mechanisms of the encapsulation phenotype.

23-137

ODOUR-GUIDED HOST FINDING BY HAEMATOPHAGOUS MOSQUITOES: OLFACTORY BASIS OF ANTHROPOPHILIC AND ZOOPHILIC BEHAVIOUR.

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Sibling species of the *Anopheles gambiae* complex show different host preferences. *An. gambiae* s.s., the main vector of malaria in Africa, is the anthropophilic member of the complex, *An. arabiensis* is opportunistic, biting both man and cattle, while *An. quadrimaculatus* is reported as zoophilic. Host-seeking is primarily mediated by olfaction. The present study aims at determining whether or not the host choices of these species are reflected in sensitivity and specificity of peripheral odour receptors.

In *An. gambiae* and *An. arabiensis* single cell electrophysiological recordings were made from antennal olfactory neurons on application of some odour substances known as host attractants. Spike recording sites were at the bases of sensilla trichodea. In *An. gambiae* and *An. arabiensis* the following compounds were tested: 1-octen-3-ol, butanone and 3-methylphenol (cattle odours) and the fatty acids C2, C3, C4, C5, iso-C4, iso-C5, laetic acid and a mixture of long-chain acids C6 - C12 (found in human sweat) and CO₂.

The two species gave similar results. No responses to long-chain fatty acids and CO₂ were found. On stimulation with the other substances, mainly excitatory responses were recorded, both short- and long-lasting, but also inhibition or excitation followed by inhibition were found. Some neurons only responded to short-chain fatty acids, in particular to C3 - C5, and not to the other compounds tested. Other cells reacted to both types of compounds. Results will be discussed in more detail at the poster.

23-139

FORENSIC ENTOMOLOGY IN SOUTHERN ITALY: THREE CASE STUDIES.

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Three case of forensic interest regarding the estimation of Postmortem Interval by entomological data are presented. The Three cases concerning criminal investigations were performed in Southern Italy by the Entomological Lab of the Institute of Forensic Medicine at the University of Bari. For each case the Authors present a description of the remains as observed at the crime scene and a detailed description of the arthropods collected from the remains. The PMI estimation was considered primarily from the stage of decomposition of the body and then from the interpretation of arthropod succession patterns. For each case the collection of insects was performed at the discovery site and during autopsy procedures. In the first case a PMI of 5 to 8 days was established based on the presence of adult specimens of *Histeridae*, *Sarcophagidae* and mature larvae of *Crysomya Rufifacies* (3rd instar). In the second case on the remains of a corpse partially burnt larvae in different developmental stages of *Sarcophaga Haemorrhoidalis* and *Phormia Terranova* were observed; a PMI of 3 to 4 days was then estimated on entomological data. In the third case a PMI of 24 to 36 hours was defined by the evidence of a *Calliphora Vicina* II instar on the body. Details on the laboratory techniques used for each case are reported. In all cases forensic significant conclusions on PMI were obtained by entomological evidence.

23-138

URATE IN THE BLOOD-SUCKING BUG, *RHODNIUS PROLIXUS*: ANTIOXIDANT ROLE IN THE HEMOLYMPH AND REGULATION OF SYNTHESIS IN THE FAT BODY

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Heme is a powerful catalyst of the formation of reactive oxygen species. We have recently proposed that blood-sucking insects suffer an intense oxidative insult as consequence of the hydrolysis of large amounts of hemoglobin after a blood meal (Oliveira et al., J. Biol. Chem. **270**, 10893, 1995). Here we show that very high concentrations of urate (2-5 mM) are found in the hemolymph of the blood-sucking bug, *Rhodnius prolixus*. TRAP assay experiments were used to demonstrate that urate is in fact the main free radical scavenger found in hemolymph of this insect. Oxidative insults such exposure to high O₂ atmosphere or hemin injection into the hemocoel resulted in increased urate levels in the hemolymph. These elevated concentrations are due to up-regulated urate synthesis in the fat body, as its secretion is completely abolished by allopurinol. The hemin-induced stimulation of urate synthesis pathway is mediated by the concerted action of protein kinases A and C. Specific inhibition of protein kinase A potentiates the increase of urate synthesis observed following exposure of the fat body to hemin. On the other hand, inhibitors of protein kinase C prevented the hemin-evoked urate release. Taken together, our data suggest that urate is a major antioxidant protection after a blood-meal in this insect.

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23-140

MORPHOMETRIC STUDIES ON *CULEX PIPIENS* ITALIAN POPULATIONS (DIPTERA, CULICIDAE)

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Culex pipiens Linnaeus is the most important mosquito of medical importance in Italy. This species is an important nuisance factor, a vector of dog filariasis and a potential vector of arboviruses. Two biological forms are present in Italy; one is anthropophilic, autogenous and stenogamous, generally breeding in under-ground, polluted waters in urban areas, and the other is ornitophilic, anautogenous and eurygamous, generally breeding in above-ground, unpolluted waters in rural areas. The identification of the two forms is important to plan selective control actions aimed to reduce the use of insecticides. Siphonal index of larvae is one of the most used character to discriminate the two forms.

Since 1980 we studied larval morphology of the Italian populations of the so-called "urban" and "rural" forms of *Cx. pipiens*. We collected about 30 natural populations of *Cx. pipiens* in different parts of Italy. The exuviae of the 4th larval instar were mounted. Lengths (valves not included) and widths (at the base) were measured, and siphonal indices were calculated (length/width). Nearly 4,500 specimens were measured.

The means of the siphonal indices of the natural populations ranged from 3 to 4.43. The populations of the "urban form" have means < 3.5 and "rural" populations > 3.8, in agreement with results from other Mediterranean countries (Roubaux E., 1945. Bull.Soc.Path.exot., 38: 47-60; Callot J., 1947. Ann.Parasitol.Hum.Comp. 22: 380-393; Senevet G. and Andarelli L., 1954. Arch.Inst. Pasteur Algerie, 32: 36-70).

To assess the possible influence of the environmental and sexual factors on the size of siphon and on the siphonal index, we reared larvae (1,392 specimens), from field-collected eggs of the "rural form" in different laboratory conditions: 15 and 30 °C; high and low densities. Results showed that larvae reared at high temperature and in crowded conditions have shorter siphon and a lower siphonal index than larvae reared at low temperature and in low density conditions. Males (275 specimens) and females (330 specimens) did not show differences in the siphonal index in two out of three experiments, as observed in Spanish populations (Eritja R. and Aranda C., 1995. Mosq.Syst., 27: 73-77).

23-141

COMPUTER-BASED IDENTIFICATION OF THE ITALIAN MOSQUITOES (DIPTERA: CULICIDAE)

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To provide the Italian National Public Health Agencies with a practical tool for identification of the 62 species of Italian mosquitoes, we have developed a computer-based expert system. The software was created using the DELTA system and it runs on IBM PC-compatible computers running MS-DOS or MS-Windows.

The electronic key is an interactive program (INTKEY) for identifying a specimen by comparing its attributes with stored description of taxa. However, some species that are morphologically very similar can only be identified up to group level. The user can identify species by checking a maximum of 67 characters for larvae and 75 for adults and identify genera by checking a maximum of 7 characters for eggs and 12 for pupae. Using a microscope, the user enters his observations about the specimens to be identified as prompted by computer menu. Morphological characters are fully illustrated and can be selected by simply clicking with the mouse. Taxa whose descriptions do not match the specimens are eliminated until reaching the identification. INTKEY program can display the characters in order of their effectiveness at the current stage of the identification, but the user can scan character menu and select the most suitable or available character. When an identification is reached, information about distribution, biology and medical importance of the mosquito can be displayed.

The package has been field tested and works accurately. It is very useful to beginners for quick and correct identification and it familiarizes the users with all required morphological characters. It has proven to be an excellent training tool for entomologists and public health workers.

The knowledge base can be expanded and refined and we plan to extend it to include other arthropods of medical importance in Italy.

The INTKEY package (data and program) can be obtained from Istituto Superiore di Sanità, Laboratory of Parasitology, Viale Regina Elena 299, 00161 Rome, Italy, Fax. (+39-6) 44.69.823. Registration of INTKEY is necessary after a trial period.

23-143

MAGGOT THERAPY FOR THE TREATMENT OF A SEVERE SKIN INFECTION IN A PATIENT WITH GANGRENE AND OSTEOMYELITIS

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A 75-year-old male patient was suffering from gangrene with osteomyelitis in his right foot. The tissue surrounding the toes and the sole of the foot up to a depth of approximately 2 cm was heavily infected with bacteria such as *Proteus mirabilis*, *Enterococcus spec.*, *Providencia stuartii* and *Staphylococcus spec.* (coagulase positive). Although the patient has been treated with antibiotics, due to the spread of the infection, two options were offered to him: amputation of the foot or maggot therapy. After receiving his consent, larvae of the sheep blowfly *Phaenicia (Lucilia) sericata* were used for the treatment. During a period of seven months and in 48 applications, approximately 6,000 sterile larvae, 24-48-hours-old, were placed on the wound of the patient. The larvae were left for 3-4 days and removed later as 3rd. instar larvae. After 4 months of treatment, the decaying tissue surrounding the toes and on the bottom of the foot detached from the living tissue. In the last 3 months of the treatment the larvae cleaned the remaining infected tissue. As the therapy progressed, new layers of tissue developed over the living tissue and the accompanying strong odor of the decaying tissues and pain from which the patient suffered intensively, decreased significantly. By the end of the therapy, a year after the appearance of the wounds in his foot, the patient was able to walk again. Maggot therapy can be recommended in cases where antibiotics and surgery are ineffective.

23-142

CURRENT STATUS OF THE STUDY OF MYIASIS (not screw-worm) IN THE MEXICAN REPUBLIC.

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The objective of the present study is to specify which genus and species of flies (not screw-worm) are affecting as myiasis various animals in the Mexican Republic until 1992-1995. The material consisted in larvae of flies sent to The Mexico United States Commission for the prevention of Aphtose fever and other exotic diseases of animals, the larvae were sent in trial flasks with alcohol at 70° for their preservance each sample had a series of data such as origin animal species date on type of wound out which the larvae had been isolated. As for the study and identification of larvae, the method of boiling it in lactophenol as well as dehydrating it in alcohol of different grades was followed so as to accomplish its later mounting between porta and cover objects by employing synthetic resin. The results obtained up to now have been found the following genus and species of flies: *Phormia regina* in animals from the 14 states of the Mexican Republic; *Cochliomyia macellaria* from 10 states; *Phaenicia sericata*, *Phaenicia cuprina* and *Sarcophaga* sp from 9 states; *Dermatobia hominis* from 6 states; *Musca domestica* from 5 states; *Chrysomya rufifacies* from 3 states. The types of wound in which larvae had been found were mainly; another type of wound; dehorning, tailing and castration. It is conclude that in 17 states of the Mexican Republic althrough 1992-1995, 8 species of flies were found affecting mexican animals in the form of myiasis, the most frequent flie being *Phormia regina* in 14 states.

23-144

ELECTROANTENNOGRAMS, ACTIVATION AND UPWIND ORIENTATION; THE RESPONSES OF STABLE FLIES (*STOMOXYS CALCITRANS*) TO HOST ODOUR

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Electroantennograms (EAGs) were recorded from laboratory-reared male and female *Stomoxys calcitrans* in response to a range of synthetic chemicals known to be electrophysiologically-active for other biting flies. 1-octen-3-ol, acetone and 3-methylphenol, consistently elicited larger electroantennograms than did control treatments; 1-octen-3-ol was the most potent. Factors affecting EAG amplitude included carbon-chain length, functional group, position of functional group, and degree of saturation.

Take-off responses to 1-octen-3-ol, acetone and CO₂ were assessed in a wind tunnel. CO₂ and acetone increased flight activity, with response thresholds between 0.001 and 0.01% for CO₂, and 0.001 and 0.01 µg/l for acetone. At low doses, 1-octen-3-ol did not affect behaviour, but at high doses it was associated with a marked reduction in flight activity.

In-flight responses to CO₂ were assessed by video recording flies as they entered a broad odour plume in crosswind flight. For CO₂, the number of upwind flights increased with CO₂ concentration, with ~80% of flies exiting upwind at 0.1%. Flight velocity decreased with dose, from 180 cm/s in clean air to 135 cm/s at 0.1% CO₂, whereas, over the same concentration range, sinuosity increased from 0.8 %/s to 1.9%/s. Similar trends were apparent for both acetone and 1-octen-3-ol.

23-145

ODOUR-MEDIATED HOST PREFERENCE OF WEST AFRICAN MOSQUITOES (DIPTERA: CULICIDAE): A CHOICE EXPERIMENT BETWEEN HUMAN AND CALF

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Mosquito host preference has traditionally been assessed by determining the blood meal origin of freshly-fed field-collected specimens. The limits of such an approach lie in the difficulty of obtaining an unbiased sample. Moreover, it does not measure the inherent host preference, but rather what is the final, contingent, host selection. A new approach was developed to test for the role of odours in the determination of host preferences, based on a standardised field trial carried out in a village near Ouagadougou, Burkina Faso. Two odour-baited entry-traps (OBETs) were put beside one another in a choice arrangement, and air coming from two tents was drawn into and blown out of the OBETs by fans via air ducts. A man and a calf of similar mass, each concealed in one of the tents, were used as odour baits. The CO₂ concentration gradient between the two traps was taken into account in the experimental protocol. An 'index of anthropophily' was calculated for each species as the weighed mean proportion of mosquitoes caught in the man-baited trap. Species with indices greater than the 0.5 random expectation included *Anopheles gambiae* s.l. (0.96) and *An. pharoensis* (0.68), irrespective of the presence or not of a CO₂ gradient. *Culex antennatus* had an index significantly lower than 0.5 (0.25), while for the *Cx. decens* species group (0.56) the difference was not significant; for the latter the interpretation was complicated by interactions between the CO₂ concentration released and the presence or not of a CO₂ gradient. Species caught in low numbers, but whose trap distribution showed a bias towards the man-baited trap were *An. funestus*, *Mansonia africana*, *Aedes dalzielii* and *Ae. hirsutus*. Conversely, species showing a bias towards the calf-trap were *An. rufipes*, *Cx. duttoni* and *Cx. nebulosus*. *Ma. uniformis* was the only species distributing at random between the two traps. Molecular identification of the *An. gambiae* s.l. samples revealed a marked difference in trap distribution of the two members of the complex present in the study area, *An. gambiae* s.s. and *An. arabiensis*; the latter constituted 92% (*An. gambiae* s.s. 8%) of identified specimens caught in the calf-trap, but only 56% (*An. gambiae* s.s. 44%) of those caught in the man-trap.

23-147

IDENTIFICATION OF MALARIA PARASITES IN MOSQUITOES, BY PCR, FROM A STUDY AREA IN GUINEA-BISSAU

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Studies were carried out at the end of the rainy season (October-November), in 30 houses of 3 subareas in Antula (254 people). Where presence of mixed infections or presence of gametocytes in some inhabitants of these houses was detected by microscopy of Giemsa stained blood smears, blood fed mosquitoes were collected from within bed nets of the same houses, at early hours in the morning. The objective of the study was to identify malaria parasites in these mosquitoes by nested-PCR.

These mosquitoes were carefully maintained in containers with glucose wet cotton pads for 10-13 days, when dissections took place. Individual midguts were kept in lysis buffer (Tris 40mM pH8.0, EDTA 80mM pH8.0, SDS 2% pH7.2) and proteinase K (50µg/ml) aimed at PCR studies for parasite identification, carried out in the Lisbon laboratory, at a later stage.

Forty-four mosquito's midguts were analysed by nested-PCR and a 39% infection rate was found, including several mixed infections. In every house/room where infected mosquitoes were captured, gametocytes (of the *Plasmodium* species detected in midguts) were observed in blood smears of the inhabitants. A parallel, further identification of malaria parasites by PCR from blood samples from the same residents (n=52), showed a high prevalence of *P. falciparum* single infections (42%), followed by *P. falciparum* + *P. malariae* (12%), *P. falciparum* + *P. ovale* (2%), with one triple infection (2%) alone, 42% being negative.

All the infected mosquitoes were identified by PCR as *Anopheles gambiae* s.s.

Evaluation of the field work, analysis of blood and midgut infection rates per room and house will be presented. The unexpected high rate of mosquito infections will also be discussed.

This work was supported by PRAXIS XXI/2/2.1/SAU/02/94 and by EC/STD3 "Network for field research on Afrotropical malaria vectors". The expert technical assistance of Mr. Marcelino Suna Nabion (CMT, Guinea-Bissau) and Ana Maria Antunes (IHMT, Portugal) is acknowledged. Dr. Georges Snounou (St. Mary's Hospital, UK) and Prof. M. Coluzzi (Univ. La Sapienza, Italy) provided us with oligonucleotide primers for parasite and mosquito identification

23-146

PRELIMINARY STUDY OF INFECTION OF GLOSSINA MORSITANS MORSITANS (DIPTERA, GLOSSINIDAE) WITH TRYPANOSOMA BRUCEI BRUCEI GVR 35 (KINETOPLASTIDA, TRYPANOSOMATIDAE) IN MICE CD1.

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The murine model CD1/*Trypanosoma brucei brucei* GVR 35 has been used for the experimental study of trypanosomiasis (Atouguia, et al., 1995, T.R.S.H.T.M. 89: 531-533). Susceptibility of tsetse flies to this model, has not been assessed.

Different groups of *Glossina morsitans morsitans* of both sexes, from a colony kept at the IHMT, were bloodfed on mice previously inoculated with *T. b. brucei* (Atouguia, et al., 1995), at different times between day 4-24 post inoculation (PI). Flies were kept at 26°C, 70% relative humidity, subsequently fed on uninfected mice every 3 days, and dissected from the 15th to the 49th day after the infectious feed (AIF). *Glossina* mortality was high (29%) up to day 15 AIF, remaining low (5-0%) thereafter. Trypanosomes were observed in the midguts from the 15th day, and in the proboscis and salivary glands (SG) from the 22nd day, AIF onwards.

The rate of tsetse infection was 48% (23/48), however, only 12.5% had SG infections and were considered infective. Both rates were similar in male and female flies. All groups of flies feeding on mice from day 4-24 PI became infected and infective. Transmission of trypanosomes to naive mice was observed after day 22 AIF.

Infection of *G.m.m.* with this trypanosome strain, from infected mice and its subsequent transmission is a useful model for the study of trypanosome/vector/vertebrate host interactions.

Acknowledgements: to Prof. A. Grácio for the use of tsetse colony, and technical support of Mrs. Silvia Nunes, Luisa Anselmo and Teresa Casaca.

23-148

THE AMINO ACID COMPOSITION OF ANOPHELES STEPHENSI (DIPTERA: CULICIDAE) INFECTED WITH NOSEMA ALGERAE (MICROSPORA: NOSEMATIDAE) COMPARED TO AN IN VITRO SYSTEM

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The amino acid composition of infected and uninfected larvae, pupae and adults of the mosquito *Anopheles stephensi* were analyzed using an amino acid analyzer.

Light microscopical investigation of the development of *Nosema algerae* within the freshly infected first instar larvae up to the adult mosquitoes showed that there were three periods during which the amounts of meronts and sporonts increased and three periods with an increase in the amount of spores.

The appearance of meronts and sporonts of the microsporidia *Nosema algerae* inside the insects coincided mainly with the increase in the concentration of alanine, proline, threonine, histidine and additionally of tyrosine during the development of the sporonts, whereas the amount of alanine and proline decreased during the sporulation of *Nosema algerae*. Tyrosine appeared in the hemolymph of infected *Anopheles stephensi*, this amino acid could not be found in the blood of uninfected insects. The changes in the amino acid composition of infected *Anopheles stephensi* were compared to those in the cell medium RPMI 1640 during the cultivation of infected cells which were derived from *Aedes albopictus*. As a result the in vitro and in vivo systems showed that the amino acid alanine and proline were important for the development of meronts and sporonts of *Nosema algerae*, whereas tyrosine seemed to induce the sporulation of the microsporidia.

23-149

RESERVES AND FLIGHT ENERGY IN FEMALE MOSQUITOES (DIPTERA: CULICIDAE)

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Three sources of energy are available to female mosquitoes for flight activities: reserves carried over from larval instars, feeding on nectar and plant juices, and blood meals from a vertebrate host. Quantitative analyses have been carried out to compare the nutritional status with the flight performance in *Aedes aegypti*, a vector of yellow fever, and in *Ae. vexans*, a holarctic species. Females were flown to exhaustion on flight mills and analyzed for lipid and glycogen before and after flight respectively. The data were correlated with flight duration and distances that were recorded from the flight mills by computer.

Development of flight performance differed between the two species. In *Ae. aegypti* it reached maximal values within 3 days of eclosion, while in *Ae. vexans* it took 1-2 weeks. These differences are explained in terms of glycogen and lipid reserves of teneral females, high in *Ae. aegypti* and much lower in *Ae. vexans*. This delay in *Ae. vexans* is caused by feeding carbohydrates to increase its lipogenesis and glycogenesis, before extended flight activities are initiated. Carbohydrates are the primary flight substrates in both species, but older females of *Ae. aegypti* divert increasing segments of glucose towards lipogenesis instead of glycogenesis.

Starvation, i.e. access to water only, drastically reduced the flight potential. Furthermore, small-sized females of both species showed equally reduced flight activities because their dietary sucrose was utilized primarily for reserve synthesis.

Ingestion of a blood meal in *Ae. aegypti* suppressed flight activity and flight potential for 1-2 days but later it was resumed, comparable to sugar-fed females.

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23-150

A POPULATION STUDY OF INDOOR *LUTZOMYIA OVALLESI* AND *LUTZOMYIA GOMEZI* (DIPTERA: PSYCHODIDAE): RELATIVE VECTORIAL IMPORTANCE AND ASSOCIATION WITH INCIDENCE OF CUTANEOUS LEISHMANIASIS IN NORTH-CENTRAL VENEZUELA.M. D. Feliciangeli¹, J. E. Rabinovich²¹ Facultad de Ciencias de la Salud, Universidad de Carabobo, Maracay, Venezuela and Escuela de Malariología, M.S.A.S., Maracay, Venezuela -² Departamento de Investigación, Universidad de Belgrano, Buenos Aires, Argentina.

In the north-central area of Venezuela *Lutzomyia ovallesi* seems to be the main vector of cutaneous leishmaniasis (CL) with *Lu. gomezi* being apparently a subsidiary vector; the former has been found to be dominantly infected with *Leishmania braziliensis* (1.2%) and more rarely with *Le. mexicana* (0.07%) and the latter infected with *Le. braziliensis* (0.47%).

Based on the conversion of 12-hours/night Shannon traps collections to 12-hours/night CDC indoor figures by linear regression, which proved to be statistically significant for both species, the results of the various non-continuous surveys during 4 years were standardized to the average number of indoor female sandflies per house per night.

Crosscorrelation analyses were then applied to female *Lu. ovallesi* and *Lu. gomezi* abundances and monthly precipitation time series. This also proved to be highly statistically significant with a lag of seven month for *Lu. ovallesi* and with a lag of six months for *Lu. gomezi*. An overall dominance of *Lu. ovallesi* over *Lu. gomezi* was observed. Since annual CL information was available for the study period, the rate of new cases per house per year was calculated and a relationship between indoor sandfly abundance and incidence was attempted by linear regression. For *Lu. gomezi* the regression was not significant while the one for *Lu. ovallesi* was highly significant showing a negative intercept. This result implies that, on the average, unless about 800 or more indoor female *Lu. ovallesi* sandflies (estimated with CDC traps) enter each house per year, we should not expect new CL cases at El Ingenio.

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23-151

CATTLE ON GRAIN DIETS IN FEEDLOTS: ITS EFFECT ON THE EXCRETION PROFILE OF IVERMECTIN IN CATTLE FAECES AND ON SURVIVAL AND REPRODUCTION IN THE SCARABAEINE DUNG BEETLE *ONTHOPHAGUS BINODIS* THUNBERG (COLEOPTERA: SCARABAEIDAE)I.R. Dadour, D.F. Cook¹

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Feeding cattle with ivermectin followed by feeding cattle with dietary supplements of grain before market is becoming a more wide spread practice in Australia. Many farmers involved in this practice have noticed a minimal amount of dung beetle activity in the dung produced by cattle in feedlot situations.

When the dung from both grain (feedlot dung) and pasture fed cattle was assessed as a food and reproductive resource for the dung beetle *Onthophagus binodis* Thunberg by bioassay in the laboratory there was a significantly greater number of F1 progeny survive to adulthood from brood masses produced by beetles reared on pasture dung compared with grain dung. The progeny emerging from brood masses produced with pasture dung were significantly larger compared with progeny emerging from brood masses produced with grain dung.

Furthermore, in this study over 5 times higher levels of ivermectin were excreted in the faeces of grain-fed cattle compared with pasture-fed (grazing) cattle. Peak excretion levels of 0.36 mg/kg (grain-fed) and 0.09 mg/kg (pasture-fed) of ivermectin were recorded at 6 and 8 days post-injection (dpi) respectively. The impact of diet on the excretion profile of ivermectin is discussed in the context of studies attempting to demonstrate ecotoxic effects of ivermectin residues in cattle faeces on dung beetles.

23-152

INTERSPECIFIC HYBRIDIZATION OF FLEAS AND ITS SYSTEMATIC SIGNIFICANCERui-yu YE, Jin-tong ZHANG¹, Zi-jian ZHANG, Xin YU, Xinru CHEN, Gen-yuan JIN and Zhong-da GONG²

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A series of interspecific hybridizations including the direct cross, reciprocal cross, the filial inbreedings and the backcross, has been made with *Neopsylla teratoma* Rothschild, 1913 and *Neopsylla sibirica* Ye et Yu, 1993, two morphologically confirmed species, as the parents. A plate-wet filter paper method was employed to get the naked pupae, which made it possible to avert the intraspecific hybridization. The morphology of both the telomere and the bristles on the horizontal arm of st.9 of the male fleas and the bristle order of st.8, were employed as the criteria for the bred progeny.

The crossability and cross-compatibility have been proved between the two species by direct- and reciprocal crosses, and the morphological intermediate types have been observed in the F1 and F2 of direct cross, F1 of reciprocal cross and in the backcross progeny. It means that the reproductive isolation has been broken, implying the parents are two subspecies under the same species with reference to the specific definition.

In view of the fact that the systematics of Siphonaptera (and also other orders of insects) is mostly based on the morphology, it's hard to completely avoid the subjective activities and voluntary treatments, thus the systematic methodology and the criteria of Siphonaptera should be improved with genetic and modern bio-techniques.

23-153

OBSERVATIONS ON BLOOD-FEEDING OF THE GROUND SQUIRREL FLEA *CITELLOPHILUS TESQUORUM SUNGARIS* (JORDAN, 1929) (SIPHONAPTERA: CERATOPHYLLIDAE)

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Ground squirrel flea *Citellophilus tesquorum sungaris* is the most important vector of plague of the ground squirrel *Citellus dauricus* Pallas in the grassland of the Northern Provinces of China. It displays high host specificity in field. *C. dauricus* is the prime host for *C. t.sungaris*.

Our experiment has demonstrated that this flea not only prefers to ground squirrel but also to Norway rat, guinea pig, clawed jird and human. The higher blood-feeding rates are on these hosts. The average blood-feeding rate on human is 80.03 (70.02-87.24)%. There are different local skin reactions on the experimental voluntary subjects to be bitten by this flea. The reactions may be divided into three types: hyperemic macule, papulation and wheal respectively.

The blood-feeding rate of the new unfed fleas depends upon the days emerged from the pupae. In five days, the blood-feeding rate shows to be increased with the lifetime (days) of fleas. There is a linear relationship between them and the correlation coefficient r is 0.95.

23-155

STUDIES ON THE HOST PREFERENCE OF MOSQUITOES AND IDENTIFICATION OF MOSQUITO BLOOD MEAL BY HEMOGLOBIN CRYSTALLIZATION IN KOREA

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Blood-meal identification of mosquitoes by the hemoglobin crystallization test have been carried out to determine the host preference in Chollanamdo province which is one of the endemic area of Japanese encephalitis and none endemic Kyonggi province in Korea. Standard models of the crystals were primarily established from the blood samples of common vertebrates and domestic animals in the residential areas.

Mosquitoes fed on variety of animals such as cattle, dog, swine, rabbit, rat, chicken and human beings. The results of hemoglobin crystallization tests for the 241 engorged females of *Culex tritaeniorhynchus* collected from Japanese encephalitis endemic area in Chollanamdo province indicated that 116(48.1%) females had fed on cattles, 34(14.1%) on dogs, 21(8.7%) on swines, 15(6.2%) on men and chickens, 13(5.4%) on rats, 2(0.4%) on rabbits and 25(10.4%) on unidentified animals, respectively.

Anopheles sinensis as a vector species of *Plasmodium vivax* malaria in Korea primarily preferred to feed on the cattles(67.8%) and dogs(32.7%) in Kyonggi province and cattles(51.1%), dogs(18.2%) and human(8.9%) in Chollanamdo province, respectively.

Aedes vexans as a vector of *Dirofilaria immitis* preferred to feed on cattles(56%) and dogs(23.9%) than other animals, and *Culex pipiens* showed preference in feeding on cattles and human in both areas.

23-154

HISTOPATHOLOGICAL & FINE STRUCTURAL CHANGES IN MOUSE SKIN AFTER INJECTION OF HONEYBEE AND SPIDER VENOMS

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Histopathological and fine structural changes in the mouse skin after injection of venoms extracted from the honeybee (*Apis mellifera*) and 3 kinds of spiders (a wandering *Pardosa astrigera*, a hibernating *Araneus* species, and orb weaving spider, *Nephila clavata*) were studied with histochemical and electron microscopical techniques respectively.

After injection of the honeybee venom, only a slight edema and contraction of epidermal tissue appeared, and this kinds of pathologic responses diminished one hour after injection, and the wounds were totally healed within 3 days at most cases. By treatment of the spider venoms, not only severe histopathological symptoms such as edema, hyperemia, eruption and necrosis, but numerous fine structural changes were induced concurrently.

It was clear that the mixtures of venom with digestive fluid had more toxic effects than pure venoms extracted from the venom gland alone. So morphological evidences are presented for the role of the digestive fluid creating additional histopathological and fine structural changes.

23-156

SARCOSAPROPHAGOUS INSECTS AS FORENSIC INDICATORS IN SOUTHEASTERN BRAZIL

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The study of the arthropod fauna associated to human remains is very important, for it provide usefull information on causes and circumstances of death. However, very little is known about this subject in the Neotropical Region. A study was undertaken in association with the Medicolegal Institute in Campinas city, Southeastern Brazil in order to determine the arthropods found or in human corpses. Six decomposing cadavers found in different sites and circumstances and live and dead adults and immatures of arthropods found in and on the remainings were collected. The live immatures were reared up to adult to make identification easier. Several especimens belonging to the orders Diptera, Coleoptera and Hymenoptera were collected from four corpses. The two remaining cadavers, one recovered from a body of water and the other burried in cement, did not have any arthropod. Through the results, the post mortem intervals were estimated and they were similar to those obtained by other methods.

23-157

URBAN POPULATION OF THE BLOOD-SUCKING MOSQUITOES

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Urban population of blood-sucking mosquitoes consist of species with wide ecological valency and those species, which are adapted to urbanized sites and conducted a transition to synantropical way of life. These species form a main core of urban mosquitoes populations in insect fauna: *Culex p. pipiens* L., *Cx.p. molestus* Forsk., *Anopheles maculipennis* Mg., *An. messeae* Fall., *Aedes flavescens* Mull., *Ae. dorsalis* Mg., *Ae. caspius dorsalis* Mg., *Ae. cantans* Mg., *Ae. vexans* Mg., *Ae. leucomelas* Mg., *Culiseta annulata* Schr., *Mansonia richiardii* Fic.

Mosquitoes *Culex p. pipiens* and *Cx.p. molestus* are absolute dominants in cities and towns of Russia. They follow man in his advance in the new developing territories. Mosquitoes *Cx.p. molestus* have passed completely to endophyllous way of life, adapted to provisional pools in basement cellars of buildings. The species of *Cx. pipiens* became endophyllous, and previously it was typically exophyllous species of low-developed territories. Its' amount has been increasing rapidly in the suburbs of cities and towns. The massive increase of these populations is caused by a human settlements growth and deterioration of sanitary conditions. The anthropophyllous population of mosquitoes are noticed in displaying the following tendencies: activity period of imago and larval populations density increase, acceleration of ontogenesis period, generations' number growth. All the above mentioned features cause the aggravation of epizootological and epidemic situation.

23-159

PHENOLOGY OF ANOPHELES SUBPICIUS GRASSI, AN UNUSUAL VECTOR OF MALARIA IN THE THAR DESERT, INDIA

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Anopheles subpictus Grassi, a major vector of malaria in Indonesia as well as in the Coromandel coastal areas in the south-eastern Indian mainland, is one of the highly prevalent anophelines in India. In the Thar desert, which is currently under the impact of vast physiographic metamorphosis due to extensive canalization, co-exists with other two main malaria vectors, *An. stephensi* Liston and *An. culicifacies* Giles. It occurs only between June and November, and is essentially a monsoon species. It thrives most at an ambient temperature $28 \pm 2^\circ\text{C}$. In typically desert conditions, it breeds prodigiously in open ground ponds, cement tanks, pitchers and the grassy banks of irrigation channels where water gets stagnated. A zoophilic species, it's anthropophilic index is estimated as 13.4. While in the highly irrigated Ganganagar district and parts of Jaisalmer district, its per-man hour relative density is about 20-50 between the period of its occurrence, in the xeric condition of Jodhpur district the relative density is 5-10 PMH only. A nocturnal species, it feeds intensely in two peaks of 8-10 pm and 4-6 am. Three females collected during routine dawn and the all-night man bait collections in some villages at the border of Jaisalmer and Jodhpur districts and under extensive canalized irrigation from the Indira Gandhi Canal, one of the largest canal systems of its kind in a desert ecosystem with over 8000 km network of tributaries and 649 km long canal, are incriminated (0.47%).

23-158

EPIDEMIOLOGICAL STUDIES OF MALARIA IN RELATION TO VECTORS IN CALCUTTA

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Malaria is endemic in Calcutta. The Anopheline fauna of the Calcutta Municipal Corporation (CMC) area has not been investigated systematically in the recent past under changed urban ecological conditions. In this study we analysed the incidence of malaria cases registered in the Directorate of Health Services (Malaria) West Bengal, Calcutta between 1987 (Total cases 16337) and 1993 (Total cases 18702) in order to find out the transmission pattern of the infections amongst the human populations. Mosquito collections were also made from different places of the CMC area and from different biotopes with a view to know the prevalence of Anopheline fauna. The anopheline fauna is composed of six species of which *Anopheles stephensi*, *An. annularis* and *An. subpictus* are labelled as primary and/or secondary vectors of malaria in India. For the last few decades, the investigators have been referring *An. stephensi* as the only vector of malaria in Calcutta albeit no systematic and indepth study has been done to assess the vectorial potentiality of the other two named anopheline species. Clinical manifestation of the infections were observed in all the years, encompassing all the meteorologically distinguishable three seasons with a pronounced peak in the rainy season (July to October). These observations suggest that there is perennial transmission of the disease and fluctuations in the level of the circulation of the parasites in its maintenance cycle.

23-160

LABORATORY BIOLOGY OF FOUR SPECIES OF CARRION BREEDING CALLIPHORIDAE (DIPTERA) IN SOUTHEASTERN BRAZIL

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The decomposition of animal carcasses occurs, in addition to other facts, through the action of necrophagous insects. In Southeastern Brazil four blowfly species *Chrysomya albiceps*, *Ch. megacephala*, *Ch. putoria* and *Cochliomyia macellaria* are considered the most important carrion-breeders. This work determined the growth curves, developmental times for egg, larva and pupa, and fecundity and mortality rates for the species reared at 13, 17, 20, 25, 28, 31 and 35°C. All experiments were done in growth chambers with constant photoperiod and humidity. Larvas were fed artificial insect culture medium and were weighed at 6, 12 or 24 hour intervals, dependig on the age. The results showed that the temperature had significant influence in all aspects studied. The interval between 25°C and 28°C was found to be the most adequate temperature for the development of these species. At 13°C eclosion of the eggs did not occur, and at 35°C the time of development was greatly reduced. However, a high rate of mortality and a low number of eggs per female were observed. These results are important subsidies that can be used in Forensic studies in Brazil.

23-161

USING *Chrysomya albiceps* (DIPTERA: CALLIPHORIDAE) TO ESTIMATE THE TIME OF DEATH IN HUMANS: A STUDY OF FOUR CASES IN SOUTHEASTERN BRAZIL

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The use of carrion-breeding insects in forensic investigations is a common practice in several countries where the basic biology of the necrophagous insect fauna is well-known. In a preliminary study, *Chrysomya albiceps* was the most abundant species visiting and breeding in pig carcasses exposed in the field. This work determines the patterns and times of development for egg, larva and pupa of *C. albiceps* reared at 13, 17, 20, 25, 28, 31 and 35°C. The experiments were done using growth chambers with constant photophase and humidity. The rate of development showed it to be highly dependent on the temperature. At 13°C and 37°C the larval development was not completed. The most adequate temperature range for the complete development of this species was between 25°C and 28°C, with larger numbers of eggs by female. The results were used to estimate the post-mortem interval of four human remains found naturally infested with *C. albiceps* immatures.

23-163

TICKS (ACARI: IXODIDAE) IN MONTENEGRO (YUGOSLAVIA) WITH EMPHASIS ON THE SPECIES *HYALOMMA SAVIGNYI*

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The faunistic composition, the relative abundance, population dynamics and the sex ratio of four tick species from the *Ixodidae* family, namely: *Ixodes ricinus*, *Rhipicephalus bursa*, *Rhipicephalus sanguineus* and *Hyalomma savignyi*, found in sheep, cattle and dogs are given in the present work.

The species *Hyalomma savignyi* is significant as vector and reservoir of viruses of CCHF and HF with renal syndrom.

23-162

SANDFLIES (DIPTERA, PHLEBOTOMIDAE) OF ENDEMIC FOCUS OF VISCERAL LEISHMANIASIS IN MONTENEGRO YUGOSLAVIA

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The endemic focus of visceral leishmaniasis in Montenegro is town Bar and surrounding. In this area the most abundance is the species *Phlebotomus neglectus* (major) - vector of kala-azar, followed by *Phlebotomus perfiliewi*, *Phlebotomus papatasi* and *Sergentomyia minuta*. The faunistic composition, the relative abundance, population dynamics, sex ratio and origin of blood meals are given in the present work.

23-164

HETEROCHROMATIN VARIATION IN THE SEX CHROMOSOMES OF SPECIES A OF THE ANOPHELES DIRUS COMPLEX IN VIETNAM.

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Anopheles dirus is considered as primary vector of human malarial parasites in Vietnam and neighbouring countries. As other vectors of human malaria they belong to complex species which difficult to identify by classical morphology. Studies on the amount of constitutive heterochromatin in mitotic karyotypes have proven useful for separating closely related species of oriental *Anopheles*.

Cytological examination of F1 larval mitotic chromosomes from a total 179 families of *An. dirus* species A from southern Vietnam population have revealed polymorphism in the sex chromosomes with respect to the amount of constitutive heterochromatin they contain.

Five variations including three forms of X and two forms of Y chromosomes have been identified in wild population samples.

23-165

IMPACT OF AN INSECT GROWTH REGULATOR AND BIOLOGICAL INSECTICIDES ON THE LONGEVITY OF THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA CARPOCAPSAE* IN MANURE

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An infective Swedish strain of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) failed to control the common housefly, *Musca domestica* (Diptera: Muscidae), in cattle production premises. A series of experiments to investigate the tolerance of the nematode to matters related to animal production premises was carried out. The longevity of the nematode when exposed to manure from cattle as well as swine was assessed. Additionally, the tolerance of the nematode to an insect growth regulator (IGR) (diflubensuron) and two commercial formulations of *Bacillus thuringiensis*, var. *israelensis* and H-14, respectively, was assessed. Animal faeces severely affected the nematodes' longevity over a 72-hour-period. When exposed to cattle faeces 12% of the nematodes survived, while exposure to swine faeces led to only 5% survival. However, the IGR did not severely affect the nematode as 79% survived. The *B. thuringiensis* formulations were highly lethal to the nematodes already within the first 24 hours. Due to its sensitivity for animal faeces, the investigated strain of *S. carpocapsae* was concluded not to be a candidate biological control agent for use in pest management of houseflies in animal production units.

23-167

SUN SCREEN INSECT REPELLENT FORMULATIONS FOR PROTECTION AGAINST MOSQUITOES

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Often the insect repellents reduce the efficacy of sun screens whereas sun screens reduce the longevity of protection of insect repellents when used separately. Sustained-release sun screen repellent formulations with three different delivery mechanisms containing 20% or 30% diethylmethylbenzamide (deet) were evaluated in the laboratory, using volunteers, for repellency against the mosquitoes *Aedes aegypti* (Linn) and *Anopheles stephensi* Liston. The sun protection factor (SPF) in the above formulations varied from 15 to 30 SPF.

The combined sun screen insect repellent formulations provided extended repellency against mosquitoes. This study showed that the repellency was not directly related to the deet concentration in the various formulations and there was no difference in longevity of protection provided by the formulations containing 20% or 30% deet.

23-166

EXPERIMENTAL INDUCTION OF SUMMER MASTITIS BY BACTERIA-EXPOSED *HYDROTAEA IRRITANS*

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Up to now, evidence of the role of *Hydrotaea irritans* to transmit the causative bacteria of summer mastitis, an acute suppurative infection in the udders of heifers and dry cows, has been circumstantial. The vector role of the fly was investigated by experimental infections of recipient heifers exposed to *H. irritans* fed with summer mastitis pathogens. Flies with verified bacterial containment were exposed to teats of eight heifers. Two teats of each animal were subjected to lesions before fly exposure. Two heifers developed summer mastitis in the quarters where teats had been scarified. The bacteria species isolated from these quarters corresponded to those that had previously been fed to the flies. Additionally, some of the pathogens were recovered from fly-exposed quarters without clinical sign of summer mastitis.

In the present study it is, for the first time, demonstrated that *H. irritans* is capable to transmit summer mastitis pathogens and cause clinical summer mastitis on recipient cattle. Lesions on the teat orifice may be a predisposing factor to develop the disease by fly transmission of relevant pathogens. Still, this mode of transmission is probably only one of many for the disease.

23-168

GLOBAL CLIMATE CHANGE: A RESEARCH CHALLENGE FOR APPLIED ENTOMOLOGY, WITH SPECIAL REFERENCE TO MEDICAL AND VETERINARY ENTOMOLOGY

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A Task Group on behalf of WHO, WMO and UNEP, working closely with Working Group II of the Intergovernmental Panel on Climate Change (IPCC), has recently revealed overwhelming evidence of climate-induced triggers in the emergence, re-emergence and resurgence of several infectious human diseases. Many of these diseases depend on insect vectors. Unstable and stable malaria, and dengue disease, now appearing at higher than ever altitudes in tropical mountain areas represent typical examples.

Global climate change forms part of a wider spectrum of human-induced environmental changes, several of which affect population dynamics of insects and disease reservoir species. Ecosystems to be most severely stressed by climate change include coastal zones, large marine ecosystems, agro-ecosystems and forests. Therefore, applied entomologists in the medical, veterinary, agricultural and forestry sectors should join current interdisciplinary impact assessment and monitoring efforts in order to help improve modelling skills, increase forecasting precision and to assist in the design of adaptive responses.

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23-169**INITIAL STUDIES TO MAP THE WHITE GENE IN *GLOSSINA MORSITANS SUBMORSITANS* USING RAPD'S****A.S. Robinson, R.H. Gooding¹**FAO/IAEA Agriculture and Biotechnology Laboratory, A-2444
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Very few genetic markers have been characterized in tsetse flies principally because of the difficulty in rearing this species coupled with the low number of progeny per female. A female tsetse will larviposit about eight 3rd instar larvae in her lifetime. The use of DNA polymorphisms generated by PCR will enable DNA from valuable pedigrees to be screened repeatedly to identify molecular markers. As a first step in this approach it was decided to try to map RAPD markers which co-segregate with a white gene in *Glossina morsitans submorsitans*. RAPD markers have also been used to examine geographical variation between populations of *G. austeni*.

The white gene in *Glossina m. submorsitans* is X-linked and a series of pedigrees was set up in which the segregation of this gene could be followed through to the F2 generation. DNA was extracted from thoraces from individuals in the P, F1 and F2 generations. The DNA was resuspended in 60 microlitres of buffer to enable up to 60 assays to be carried out per individual. From one hundred 10mer oligonucleotide primers (UBC RAPD Set 100/1) checked for their ability to amplify DNA from *G. m. submorsitans*, 66 gave reproducible results with a varying number of bands being produced for the different primers.

Initial results of the pedigree analysis identified several primers which produced bands showing segregation with white. These will be confirmed and the analysis extended to include all 66 primers.

Section 24

Entomology for the Third Millennium - Critical Issues

24-002

QUANTIFICATION OF RISK FOR QUARANTINE SYSTEMS USING COMBINED COMMODITY INFESTATION AND TREATMENT EFFICACY ESTIMATES.

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Several approaches to determining the risk of pest introduction have been proposed based on rates of infestation of commercial commodities. Rare infestation of the commodities because of non-host status or low pest populations may justify eliminating the requirement for postharvest quarantine treatments or reducing the severity of treatments. The "probit 9" (99.9968% mortality) was proposed in 1939 by USDA entomologists based on treatments for fruit flies. The probit 9 requirement has a questionable mathematical basis and frequently is not obtainable without unacceptable damage to many commodities. Recently techniques to calculate the conditions required ensure a given level of security so as not to exceed a maximum pest limit have been proposed. We examined the risk of pest introduction for the Mexican fruit fly *Anastrepha ludens* Loew for a series host fruits and fruit production regions of Mexico. Proportions of fruit infested and numbers of insects per infested fruit were recorded for commercially important citrus and mango and for native hosts. Then the required treatment efficacy was calculated to allow less than a mating pair of Mexican fruit flies per commercial shipment. In commercial orchards when fruit fly management programs controlled pests, the probit 9 requirement was found to be slightly excessive (99.984% mortality = probit 8.86 would have been sufficient). In cases with no fruit fly control, infestation rates would require treatments far in excess of the probit 9 level (>99.9997% mortality = probit 9.55). These calculations indicate that the currently required treatment efficacy provides security against introduction of a reproductive pair of flies only in fruit production areas that are managed to control fruit flies. The current management systems do not, in general, warrant reduction in treatment efficacy requirements.

24-001

DEVELOPMENT OF A STATISTICAL APPROACH FOR COMPARING THE EFFICACY OF AREA FREEDOM, NON-HOST STATUS AND DISINFESTATION TREATMENTS AS "TREATMENTS" AGAINST FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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The essence of maintaining quarantine security is to minimise the probability of the introduction of a quarantine pest to an acceptable level, in some cases necessitating the application of quarantine treatments. The current methods for ensuring quarantine security against fruit flies that specifically address this probability involve disinfestation treatments based on either a mortality of 99.9968% (probit 9) or modified approaches such as the Maximum Pest Limit. These methods ensure that the probability of an individual or group of individual pests surviving a specific treatment application is at, or lower than, a predefined maximum limit. Other "treatments" which are used to maintain quarantine security, such as area-freedom and non-host status, have not previously been considered within this probabilistic framework. For a disinfestation treatment, the probability of a mating pair surviving can be reasonably estimated. The true error in area-freedom monitoring is the probability of an area not being "free" of a specific pest given that monitoring, which would detect with a certain probability the presence of a specific minimum infestation level, failed to detect the pest. For non-host status assessments, the true error is the probability of a fruit being capable of hosting a pest at minimum infestation levels, given that this capability is not detected. For both area-freedom and non-host status, this minimum infestation level would be set such that if the pest were present then the probability of a mating pair within a consignment does not exceed a specific, maximum level.

24-003

INTERNATIONAL TRADE NEGOTIATIONS

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Negotiations of agricultural issues between two countries who are trading partners should be based upon good science. The need for restrictions or special conditions on exporting products must be supported by biologically sound reasons. If exports of agricultural products are denied because of physical and/or cosmetic characteristics on the cartons, for example, the United States considers this a trade barrier. Several incidents involved U.S. products which were stopped because the labels on the export cartons were curved instead of straight or 2 mm too big or too small.

A more serious phytosanitary issue is the rejection and/or treatment of agricultural exports caused by a policy of "zero tolerance" in reference to finding cosmopolitan or common insects and organisms in arriving shipments. This has led to the question of whether this should be referred to the World Trade Organization when the shipment of a commodity, such as lettuce, has a rejection rate as high as 40-50%

These are problems which lead to trade barrier concerns. International trade negotiations are not only done at the tables of a formal bilateral meeting between two countries, but can and should be negotiated during on-going discussions by the representative in-country. There are a number of effective ways for conducting this.

24-004

IRRADIATION: A NEW CHALLENGE TO INTERNATIONAL TRADE IN FRUITS AND VEGETABLES

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Global trade liberalization in food and agricultural commodities is in effect following the Uruguay rounds of the GATT. Irradiation provides a new challenge to the trade in fresh fruits and vegetables by overcoming quarantine barriers against insect pests, especially fruit flies. This challenge is met through harmonized data on the effectiveness of a small radiation dose (150 Gy minimum) to provide quarantine security against major fruit fly species regardless of host commodities. These data were developed by a research programme of the Joint FAO/IAEA Division and evaluated by international experts appointed by the International Consultative Group on Food Irradiation established under the aegis of FAO, IAEA, and WHO. An international consensus on the acceptance of irradiation as a quarantine treatment was reached by regional plant protection organizations (NAPPO, EPPO, APPPC, COSEVE, OIRSA) which operate within the framework of the International Plant Protection Convention. Following the announcement of the USDA's position to accept irradiation as a quarantine treatment of fresh fruits against major fruit flies regardless of host commodities, a regulation to this effect is expected during 1996 which would open the US market to irradiated fruits, properly treated for quarantine purposes for the first time. The final challenge on the acceptance of irradiated fruits rests with the consumer. Limited commercial-scale marketing of irradiated food conducted in several countries in general and irradiated fruits in the USA in particular, has demonstrated that consumers would be prepared to accept irradiated food once they receive proper information. Thus, irradiation could expand the horizon in trade in fresh fruits and vegetables while keeping the spread of insect pests in check.

24-006

DISINFESTATION OF PESTS ON CUT FLOWERS WITH GAS MIXTURES OF METHYL BROMIDE, PHOSPHINE, AND CARBON DIOXIDE

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Each stage of eight species of arthropod pests, *Tetranychus kanzawi*, *T. urticae*, *Thrips palmi*, *Trialeurodes vaporariorum*, *Myzus persicae*, *Aphis gossypii*, *Planococcus kraunhiae*, and *Plutella xylostella*, on chrysanthemum and orchid was fumigated with gas mixtures of methyl bromide (10g/m³), phosphine (3g/m³), and carbon dioxide (5%) for 3, 4, and 6 hours at 15 and 20C. *T. kanzawi* egg was the most resistant stage and was killed completely at doses of 13g/m³ of methyl bromide, 3g/m³ of phosphine and 5% carbon dioxide with 40% (v/v) loading at 15C for 4 hours and 20C for 3 hours. Thirteen percent of the initial dose of methyl bromide was adsorbed by the cut flowers and packing materials, while no phosphine adsorption was observed. Carbon dioxide concentration increased due to respiration of the cut flowers. No injury was observed to six cultivars of chrysanthemum and four cultivars of orchid fumigated at 15C for 4 hours. A slight injury was confirmed on both cut flowers when fumigated at 20C for 3 hours, followed by storage at 15C or above. The injury, however, could be acceptable to commercial trade. Carbon dioxide in the methyl bromide-phosphine mixture is useful to prevent ignition of the phosphine and reduce concentrations of methyl bromide needed which will result in less damage to the flowers.

24-005

THE SYSTEMS APPROACH TO QUARANTINE SECURITY

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Quarantine treatments against fruit and vegetable-infesting pests employed today usually consist of some component, such as heat, cold, or fumigation, which may damage the commodity being treated. The equipment required to apply this treatment and the trained personnel to run it are added expenses to the marketing of fresh commodities. In recent years many commodities have been reported as possessing varying levels of resistance to quarantined pests. The use of plant hormones, such as gibberellic acid, has been shown to reduce the susceptibility of fruits to fruit fly attack. Packing house procedures designed to preserve shelf life often reduce the risk of pest infestation. In situations where sufficient biological and ecological information about the pest-commodity system is available it may be possible to use this knowledge to achieve quarantine security. This method is commonly referred to as the systems approach. The systems approach integrates biological, physical, and operational factors that can affect the incidence, viability, and reproductive potential of a pest into a series of procedures which together provide quarantine security. Some working examples of this technique are grapefruits in Florida, apples from the northwestern US, papayas from parts of Costa Rica and Brazil, avocados from Mexico, and nectarines from California. These successes illustrate a very promising methodology which should expand worldwide during the next millennium.

24-007

THERMOSENSITIVITY IN IMMATURE STAGES OF QUEENSLAND FRUIT FLY, *BACTROCEA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE)

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Immature stages of Queensland fruit fly comprising initially early and mature eggs, were subjected to four preconditioning temperatures (between 30-40C) for one to six hours prior to challenge with a lethal temperature of 46C at a time duration necessary to achieve 50% mortality. At the early egg stage (2 hours old), eggs acclimated at 30, 35, and 37C showed lower, but statistically nonsignificant, percentage mortality than the control group. When eggs were acclimated at 40C, the control showed significantly lower mortality. For the mature egg stage (26 hours old), similar trends occurred for acclimation at 30, 35, and 37C, but greater differences were seen between the acclimated and control, especially for the latter two temperatures. Within the 35 and 37C acclimation group, significant differences in mortality were consistently observed as the duration of acclimation was prolonged. At 40C the mortality of the acclimated group was lower than the control group for the first two hours of treatment, and the trend was reversed until the sixth and final hour of acclimation. Responses of early and mature eggs were, thus, different when subjected to similar acclimation and challenge temperatures. Variations may be due to the differences in 1) stage of egg development, 2) duration of exposure, and 3) acclimation temperature. The implications of each of these factors are discussed.

24-008**HIGH-TEMPERATURE CONTROLLED ATMOSPHERES FOR POST-HARVEST DISINFESTATION OF TEMPERATE ARTHROPODS**Diana C. Whiting

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Controlled atmospheres and heat are separately under intensive investigation as non-chemical postharvest disinfestation treatments against temperate arthropods of quarantine importance found on fresh fruit. Combining these two accepted technologies results in novel treatments capable of comparable pest kill with reduced doses of each components. In addition, high temperature-controlled atmosphere treatment combinations provide greater scope for modification to a) control pest complexes on the same host fruit, and/or b) recognise the specific treatment tolerances of different host fruit types. Further treatment flexibility lies in manipulation of the application protocol of such treatments.

Recent examples from the high-temperature controlled atmosphere research programme to disinfest New Zealand export apples, kiwifruit and stonefruit of Lepidoptera, Hemiptera, and Acari will be discussed, and possible avenues for future treatment combinations with other pest control strategies applied either before or after harvest will be highlighted.

24-009**NOVEL TECHNOLOGIES FOR QUARANTINE TREATMENTS**Guy J. Hallman

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Several events are increasing the need for new types of quarantine treatments. Ethylene dibromide was lost as a fumigant a decade ago, and methyl bromide may be lost by the end of this century or shortly thereafter. More commodities host to more pests are being transported to more countries than ever before. Improvements are sought in some treatments which have been developed to replace fumigants. The cold treatment is lengthy (two weeks or more) and causes damage to some commodities on which it is currently being used. Hot water immersion of mangoes often damages mangoes. New methods of quarantine treatment are being studied. Fruit coatings and waxes have been shown to kill surface and interior quarantine pests. Coating combines well with other fruit fly quarantine treatments, such as heat and insecticide dip. Coatings by themselves may yield considerable levels of fruit fly mortality, thus, being useful to the systems approach. Ohmic heating shows promise as a quarantine treatment. An electrical current is passed through fruit and resistance causes the fruit to heat. Fruit can be heated to temperatures sufficient to kill fruit fly immatures fairly uniformly in a matter of a few minutes. Other treatments, such as microwave heating and high pressure, are being examined in new ways which may show promise as quarantine treatments in the third millennium.

24-010**DEVELOPMENT OF QUARANTINE STRATEGIES IN LIEU OF TREATMENT--THE CASE OF THE WALNUT HUSK FLY AND STONE FRUITS EXPORTED FROM CALIFORNIA**Victoria Y. Yokoyama and Gina T. Miller

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The walnut husk fly, *Rhagoletis completa* Cresson (Diptera: Tephritidae), has been considered a pest of regulatory concern by certain countries that import stone fruits from California. Research showed that the risk of accidental introductions of the fruit fly into foreign countries was minimal based on the establishment of a pest-free period between the beginning of stone fruit harvest and July 1. The pest is not prevalent in its normal host, walnut orchards, during this period in the San Joaquin Valley of California where the highest production of stone fruit in the world occurs. The pest-free period was verified in 1990-1994 through an extensive trapping program in walnuts in five counties (Merced, Madera, Fresno, Tulare, and Kern) in the San Joaquin Valley. The pest-free period was further supported by research that showed that peaches and nectarines were not good hosts and unlikely to be infested and that plums were not a walnut husk fly host. Another quarantine strategy, low temperature fruit storage, was found to minimize risk for accidental introductions of the pest into foreign countries. Although a methyl bromide fumigation was developed as a quarantine treatment to control walnut husk fly in peaches, industry preferred the development and use of alternative control strategies. Acceptance of the research resulted in regulatory agreements that allow export of peaches, nectarines, and plums from five counties in California and supports a multi-million dollar global export market.

24-011**THE WORLD WIDE WEB AND AGRICULTURE - WHY AND WHO**

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The World Wide Web has exploded as a major source of agricultural information. This paper will examine the organizations active in promoting the Web for agriculture, a brief history, and the benefits (and shortcomings) of information dissemination on the World Wide Web.

24-012

SUSTAINABLE CROP PROTECTION INFORMATION FROM THE INTERNATIONAL AGRICULTURAL RESEARCH CENTERS

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Global Strategies for Electronic Communication of IPM Knowledge Bases. Organizers: Drs. G. Schaefer (CICP) and R. Stinner (CIPM)

Research related to sustainable crop protection forms a very significant part of the work of the International Agricultural Research Centers (IARCs) of the Consultative Group for International Agricultural Research (CGIAR). However, this work has not realized its full potential in contributing to the worldwide implementation of integrated pest management (IPM) for lack of an effective mechanism to coordinate these activities and make their results widely available. To address this need, the CGIAR System-wide Programme for IPM was launched at the beginning of 1996. An important component of the System-wide Programme will be to provide a common electronic access point to the wealth of relevant information already available and continuously being generated. In this initiative, the System-wide Programme will work closely with partners in information technology outside the CGIAR system to ensure the maximum possible compatibility and synergism with their efforts. Within this broader context, the IARCs can play a constructive role in helping to ensure that the information technology initiative as a whole is fully responsive to the needs of their IPM partners in the national agricultural research systems of developing countries and can assist directly in making relevant information fully accessible to these partners.

24-014

THE CROP PROTECTION COMPENDIUM - REGIONAL APPLICATION AND GLOBAL STRATEGY

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The Crop Protection Compendium is an encyclopaedic database resource for use on PCs, designed to provide access to information on all aspects of pests and their integrated management from a single point and in a user-friendly format. A version for South-East Asia, to be published in 1996, contains data sheets on 1000 pests, diseases and weeds of economic significance to the region, together with information about their natural enemies and host crops. For each species, biological and agricultural data are supplemented by text descriptions, illustrations, distribution maps, abstracts of the literature, taxonomic information, accounts of control, and diagnostic systems, all connected through relational links and with points of access to external databases.

A special focus is placed on IPM. The Compendium presents balanced information about the full range of control measures for each pest, including plant quarantine, cultural control, genetic resistance, biological control, and the appropriate use of pesticides. A bibliographic database of the international literature of IPM is included, with special emphasis on South-East Asia.

The Crop Protection Compendium will be published on CD-ROM. An extensive survey of user needs indicates that it will be keenly used by a wide range of pest management specialists, extension staff, quarantine officers, plant breeders, university teachers and students, crop managers, and policy makers. Users will be able to record their own in-context notes electronically and will be invited to submit these to the developers, to be screened for possible use in keeping the Compendium accurate and up to date.

The Crop Protection Compendium for South-East Asia is expected to become an influential standard resource in and beyond the region, widely used in IPM projects. It will be followed in 1998 by a version with global coverage. This will provide a strong basis for a global information system for IPM.

24-013

TED RADCLIFFE'S GOPHER STATE IPM SITE: IPM TEXTBOOK ON THE WORLD WIDE WEB

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We have launched development of an IPM textbook, Ted Radcliffe's Gopher State IPM Site (URL: < <http://www.ent.agri.umn.edu/academics/classes/ipm/ipmsite.htm> >), on the World Wide Web (Internet). This project is an initiative of the University of Minnesota and co-sponsored by the Consortium for International Crop Protection (CICP) and the National IPM Network (NIPMN).

As of 1 January 1996, more than 100 US and internationally prominent entomologists have agreed to contribute chapters on various aspects of IPM. We intend to expand coverage to include all pest management disciplines. We invite contributions (3,500-5,000 words, with 5-7 color photographs and 5-10 key references) from IPM specialists around the world. There is no limit on the number of chapters we can post and different perspectives on the same topic are desired. We will initiate a system of peer review of contributions and to leave material posted on the server for 2 years. Chapters can be updated or completely revised whenever the authors wish.

Our purpose in constructing this site is to provide: 1) a venue for easily maintaining and up-dating "state of the art" information from the world's leading experts on all aspects of IPM, 2) a resource economically deliverable anywhere in the world that can be freely downloaded and used by students, teachers, and IPM practitioners, 3) a forum for the international presentation of practical information and theory on IPM, 4) links to the vast and rapidly growing IPM resources available on the Internet including photographs, and decision-support software.

24-015

THE NATIONAL IPM NETWORK - ONE ANSWER TO THE INFORMATION GLUT ON THE INTERNET

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The National IPM Network (NIPMN) in the United States was established as a grass-roots effort to provide electronic dissemination of pest management information to diverse audiences. NIPMN includes a national server and four regional servers linked with many state servers.

24-016

IPMNET, A RESPONSE TO THE CRITICAL NEED FOR INTERNATIONAL IPM INFORMATION/COMMUNICATIONG. Schaefers

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Global Strategies for Electronic Communication of IPM Knowledge Bases
Organizers: Drs. G. Schaefers (CICP) and R. Stinner (CIPM)

Launched on the internet by the Consortium for International Crop Protection in 1993, IPMnet was designed in response to the need for improved crop protection strategies of less developed countries. It was launched on the Web in 1995 (<http://ipmwww.ncsu.edu/cicp/cicp.html>). It is currently operating with a search mechanism, registration database, various information databases, an interactive bulletin board, and the monthly IPMnet News. Plans are underway to develop a more global approach through partnerships with other international IPM systems. The Consortium is convinced that its emphasis on electronic communication can serve as a cost effective, primary mechanism for the cooperation and collaboration that is needed to increase the rate of development and adoption of effective IPM in developing nations.

24-018

IPM WORKING FOR DEVELOPMENT: INTERNATIONAL COOPERATION THROUGH THE INTERNETC. Addison/M. J. Iles

European IPM Working Group and the International IPM Forum, NRI, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK.

Global support for information exchange and regional and local initiatives for the promotion and implementation of integrated pest management.
Secretary: Mr Malcolm Iles (NRI).

The European IPM Working Group server started in 1994 has recently developed a WWW site (<http://www.nri.org/ecart.htm>) serving the European Research Community with database information on Project activities in IPM and a guide to Institutional and Professional resources. This forms part of a wider initiative supported by the International IPM Forum to make newsletters and publications freely available through the Internet and disseminate IPM information in disk CD Rom and hard copy form to those without Internet access.

24-017

AFRICALINK AND OTHER INITIATIVES OF THE U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT (USAID) TO FACILITATE IPM INFORMATION NETWORKING IN AFRICA AND WORLDWIDE
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USAID is engaged in a number of activities to facilitate access to the Internet and other electronic communications media for its colleagues and partners. Worldwide, public access to electronic mail connectivity is least developed in Africa: some 15 countries lack even the most basic access. A specific initiative to address this need is AfricaLink, in which the focus is on the end users of technical information, such as for crop protection. Within the context of each country's existing infrastructural and regulatory environment, AfricaLink implements simple strategies to help end users obtain electronic mail access as quickly as possible. As a general strategy, AfricaLink encourages networks to obtain electronic mail access through a local service provider.

USAID's IPM Collaborative Research Support Project is working with AfricaLink to connect scientists, NGOs and other users and developers of IPM information in African countries. It is also establishing networking connections among IPM researchers and practitioners and colleagues in the Caribbean, Central and South America, Eastern Europe, and Southeast Asia, with the immediate goal of promoting more efficient collaboration with U.S.-based colleagues. This process is being facilitated through cooperation between the U.S. National IPM Network and the internationally-oriented IPM Net of the Consortium for International Crop Protection. Case studies will demonstrate how a two-fold purpose is being promoted, to (a) transfer appropriate information technology and strategies, while (b) strengthening indigenous crop protection capacities in developing countries.

24-019

A CLASS TO ENABLE TEACHERS TO USE INSECTS TO FACILITATE LEARNING BY ELEMENTARY STUDENTS.A.C. York

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In the Entomology Department at Purdue University I have created a one-credit, two-hour per week laboratory course designed to teach future and current teachers how to use insects in the classroom as learning tools. Because we believe that most children have an inherent interest in and curiosity about insects, they are an ideal classroom learning aid. They are effective as tools for teaching not just science and math, but social studies, language arts, human hygiene, and management of insect pests. The course uses a group of insects readily available to most teachers and easily and conveniently maintained as classroom "pets." The students in this course become extremely familiar with this group of insects and feel comfortable handling, raising, and using them to achieve desired educational objectives. Moreover, should a teacher decide to teach a unit about insects, the material learned in this class provide everything needed to easily do so. In addition to achieving its goals, this class has achieved additional success in that students completing this class frequently enroll in additional classes in entomology: introductory entomology, insect behavior, apiculture, or spider biology.

24-020

"GETTING A BUZZ FROM INSECTS" - AN AUSTRALIAN PROGRAM FOR ENCOURAGING INSECT EDUCATIONAL ACTIVITIES IN SCHOOLS.

G.B.Monteith & H.A.Janetzki

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Insects have much to offer school curricula. They are attractive, diverse, versatile, easy to breed, short lived, available everywhere and usually free. School children come to public museums for advice and assistance in undertaking school projects with insects. In fact they often come in such large numbers that staff can't cope. We found that a major reason they come is because their teachers have very poor knowledge about insects and the ways to use them in the classroom.

To try to achieve the dual result of reducing school children demands on staff time and improving teacher skills with insects, we devised an intensive 3-hour hands-on workshop for the teachers themselves. To break down the powerful cultural inhibition against touching "creepy crawlies" we gave them the opportunity to handle about 25 species of living insects, ranging from mealworms to locusts, caterpillars, phasmids and giant burrowing cockroaches. In an atmosphere of fun we led them through the evolutionary success story of Insects and on to catching, preserving, breeding, modelling and experimenting with them. We stressed the improvisation of apparatus (nets, pinning boards, cages) from spare materials so that teachers could themselves put into practice every activity we taught them. They received a resource kit of printed information and other ideas.

The workshop proved immensely popular among teachers. Sessions at the Museum in Brisbane were booked out when offered. Later by gaining sponsorship assistance from commercial traders (a Butterfly House and a retailer of insect apparatus) we were able to tour the workshop around Australia. This tour was linked with separate tours of travelling insect exhibitions. The workshop has now been presented 104 times in 25 centres around Australia and has given insect skills to almost 2,000 teachers.

This workshop program has had a significant effect on the level of insect awareness in Australian schools and provides a simple formula which many other Museums could follow.

24-022

NONTRADITIONAL LEARNING OF ENTOMOLOGY

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Entomology and entomologists continue to suffer from an image problem. Many people characterize entomologists as slightly eccentric, a bit weird, and downright strange because of the subject they study. Movies and other media support this stereotype through the dissemination of improper materials. Entomologists need to inform the public of the wonders of entomology and the important role that entomologists play in their everyday lives. While classroom education can help, only a small fraction of people are reached and educated. Young people have an innate fascination for insects and their arthropod relatives but adults, through their own negative attitudes toward insects, overtly or subliminally discourage further exploration into entomology. Entomologists have the opportunity to educate a wide audience through non-traditional educational. Bug Bowl, presented by the Department of Entomology at Purdue University, has been a spectacular success reaching 7,000 people over a two-day period. Other successful, non-traditional programs are presented.

24-021

INSECTS FOR CHILDREN

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Children have a natural affinity for the diverse and intriguing world of insects. We have developed and participated in programs that bring the wonderment of entomology to children in both classrooms and in natural settings. Examples of programs include: 1) Life in A Pinecone; this program was developed using Berlese funnels to collect insects from pine cones after seed release. New species of insects collected included species of Psocoptera (barklice). An educational module was developed for collecting insects from pine cones; 2) The Monarch Butterfly's Marvelous Migration; the ecology and migration of the monarch butterfly, *Daneus plexippus* L., were demonstrated in both in-class presentations in New Hampshire and Texas; and in a poster sent between schools. The poster was later expanded and titled "Butterflies 'n Beetles;" the final poster on insects was 45 cm wide and 1000 m long. It was displayed in the Smithsonian Institution Museum of Natural History in Washington, D.C. The project has been expanded across the United States; 3) Outreach Programs Using Insects in the Classroom; as part of regional and national meetings and local volunteer efforts, insects and insect displays are brought into classrooms and to exhibit areas. One activity included coordination of Insect Expo '94 through the Entomological Society of America national meeting in Dallas, TX. Over 50 presenters and 3500 youth interacted with insects displays, presentations and insect lore. These three examples are ideas for entomologists using insects in their presentations.

24-023

PREPARATION OF ENTOMOLOGY STUDENTS FOR A CAREER IN THE AGRICHEMICAL INDUSTRY

F.D. Tenne

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Major agrichemical companies operate global businesses. There is a need for well trained entomologists to enter into these companies. Students need to acquire and demonstrate competency in scientific disciplines, communications, and social skills. Excellence in these collective skills significantly influence and enhance the success potential of students as employees.

Initiatives by American Cyanamid to attract and develop students as potential employees is an ongoing process. University involvement in working collaboratively with industry to prepare students will ensure students entrance and career contributions as employees in the agrichemical industry.

24-024

TECHNIQUES AND STRATEGIES FOR TEACHING
ENTOMOLOGY TO LARGE CLASSES
F.T. Turpin (West Lafayette-United States)

ABSTRACT NOT RECEIVED

24-025

ENTOMOLOGY AND PLANT PROTECTION EDUCATION
IN SOUTH AMERICA

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Argentina

South America embodies fourteen countries with different environments ranging from plains to high mountains, and climates from tropical to polar. How to approach youth education with such insect diversity? Yet there are two big concerns: preservation of the habitat and food production for local and foreign markets. With both objectives in mind, most countries follow the traditional way of teaching basic entomology through biology departments and applied entomology through agronomy departments. A discussion is provided of entomology and plant protection education at the undergraduate and graduate levels in these countries.

24-026

ENTOMOLOGICAL EDUCATION: CAN WE REALLY TRAIN
STUDENTS TO BE HOLISTIC?
R.G. McKinlay, D. Wyllie (Edinburgh-United Kingdom)

ABSTRACT NOT RECEIVED

24-027

COMPUTER BASED IDENTIFICATION, TRAINING AND
SUPPORT IN ENTOMOLOGY

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Department of Entomology, The University of Queensland, Brisbane,
Australia.

Making knowledge accessible to all inquiring minds will be the hall mark of the next century. The lack of ready access to libraries of 'scholarly works', and the mysterious jargon in which such works are often written, has in part constrained communication even within disciplines. The advent of personal computers, linked by improved telecommunications and software, is ushering in a new age of open learning. To realise the potential these technological developments offer a discipline such as entomology requires a new approach to packaging and presenting information. In a 'hands on' workshop, we present a number of sophisticated yet user friendly software packages being developed to take entomology to a much wider audience. The software packages illustrate this new approach to problems ranging from the simple presentation of 'facts' about particular insects, to interactive identifications keys; from training students in diagnosing pest problems, to developing complex models of insect life cycles, and from analysing the distribution of a species and its potential for spread, to running simulation of insect migration over the world wide web. Specific software packages that will be demonstrated at the workshop include:

BUGMATCH - a decision support system for growers and consultants
LUCID - an interactive taxonomic key and builder
DIAGNOSIS - an interactive game which teaches diagnostic skills
GENSECT - a generic population model and builder
CLIMEX - for predicting species distribution
GENSIM - a spatial model to predict movement of migratory pests

This symposium and workshop will interest all entomologist concerned with communicating information about insects; be they teachers wanting to excite students about their science, or researchers needing to make results readily digestible to the public and even fellow scientists.

24-028

AN EXPERT SYSTEM FOR CLASSICAL BIOLOGICAL CONTROL

M.A. Golosova, F.N. Semevski¹, L.C. Thompson²

The Moscow Forestry Institute, Moscow, Russia - 1 Institute of Global Climate and Ecology, Moscow, Russia - 2 University of Arkansas, Monticello, Arkansas, USA.

This system forecasts the outcomes of classical biological control activities. The system includes a database, three groups of population and economic models, commentary, and a tutorial. The forecasts are based on analysis of historical data, experience in classical biological control, and the modern theory of population dynamics. One can select from three groups of models: First, population dynamics of the target species (pest) in its new area of residence. Second, a model representing how natural enemies would suppress the pest in its new land. Third, a model describing interactions between the natural control agent and the target species. An economic module calculates a return on investment in the biological control effort. The system also includes an adaptive feature when supplied with new information. The system may be useful for both teaching and applying biological control. The system is written in Professional BASIC, and uses 1.2 megabytes of disk memory.

24-029

A MONITORING OF SOME INSECT IN NORTH CAUCASUS BY DINT OF REMOTE SENSING METHODS AND FORECASTING EXPERT SYSTEMS

M.I. Saulich

All-Russian Institute of Plant Protection, Saint-Petersburgh, Russia

In pursuing these aims the methodical approaches have been developed: remote sensing phytosanitary diagnostics and forecasting based on expert computer systems. Remote sensing methods are oriented to application of visual observations from helicopters and large-scale air-photography from light aircrafts for discovery of microfocuses with breeding of such species as *Lema melanopus* (L.), *Zabrus tenebrioides* (Göese), *Loxostege sticticalis* (L.), larvae of *Agriotes* sp..

As diagnostic indicators, parameters of lots of vegetables damaged by insect-phytophages are used in grain, industrial and fodder crops. It is being developed models for going from damage estimations to insect densities on fields.

Expert computer systems are created using LOTUS PLUS "shell", allowing to a large degree to automate the process of base knowledge development for short-, long-term and long-standing forecastings of population dynamics of above mentioned insect species and some others.

In the end, a combination of forecasting and remote sensing diagnostics leads to a significant reduction of plant protect measures and pollution environment with pesticides, application of soft measures of struggle with consideration for environment protection interest.

A general scheme of monitoring of main phytophage distribution is illustrated for grain, industrial and fodder crops.

24-030

CABIKEY - A NEW MULTIPLE ENTRY KEY SYSTEM FOR IBM COMPATIBLE PERSONAL COMPUTERS

I. M. White

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CABIKEY (Computer Aided Biological Identification System) is CAB International's software package for interactive identification. CABIKEY is a multiple entry key, i.e. it works directly from a database of coded taxonomic descriptions, rather than through a hierarchy of ordered questions. CABIKEY was initially developed for the MS-DOS operating system and the DOS version of the software has now been used for a beetle family key (available from CABI since late 1994) and a key to European thrips pests (available very soon). Other current CABIKEY projects include mosquito genera, a group of fruit flies, thrips from other regions and two termite applications; many of these projects are joint with the Natural History Museum. A Windows version of CABIKEY is now being developed, including a special "developers" version that will allow the creation and editing of new keys. The Windows version of CABIKEY allows the user to run in a simplified mode, an advanced mode, or to choose characters (questions) almost at will. The advanced mode lists characters both by recommendation and then by a separation "score" based on the number of paired combinations of remaining taxa each character separates. The simplified mode presents characters in sequence following the recommended and score sorted lists (which remain hidden in this mode). Characters are presented as pictures with supporting text and help files. Each taxon can be allocated text, pictures and a distribution map, plus a video or sound clip. Facilities are also provided to list a tabulated comparison of two taxa and to place any two or more pictures together for comparison. The developers version will also allow import and export of DELTA and spreadsheet compatible ASCII files, the export of HENNIG files, and provide its own spreadsheet-like editing interface for the data.

24-031

A HYPERTEXT ON PREDATORS OF DIASPIDID (HOMOPTERA, DIASPIDOIDEA)

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* Istituto di Entomologia Agraria - Università di Bari - Italy.

** Dipartimento di Biologia, Difesa e Biotecnologie agro-forestali Università della Basilicata - Potenza - Italy.

Diaspidid predators belong to lots of different taxonomical groups and are currently diffused or dispersed to colonize new zoogeographical regions. That's why it's difficult to discriminate useful insects for a field technician.

A hypertext is presented, which allows identification of diaspidid predators from whole the world; it gives the following information for each species:

- 1) taxonomic position;
- 2) distribution;
- 3) bionomics;
- 4) diaspidid hosts;
- 5) diaspidid host plant;
- 6) iconography

Information are searchable either by predators name or diaspidid species or diaspidid host plants or geographical region or macroscopical characters.

24-032

APPLICATION OF MICROCOMPUTER IN AGRICULTURAL INSECT IDENTIFICATION

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The software for identification system of agricultural insect was made by FOXbase+. This software identifies agricultural insects basing on combination of morphological characters and injury characters, which makes it easy to be applied and popularized, as well to identify correctly. On screen design, user-friendly interface, novel pictures and optional menus make very easy to be learned, i.e. you can operate it by pressing simply function keys to draw a conclusion. This system contains information of 33 orders of insects, 240 families and 44 species of agricultural insects, in which there are: 1. classification of insects, 2. identification of soil insect, 3. identification of insect pests of rice, 4. identification of insect pests of cereals. The software, with function of study, was available for veteran and recruit working for plant protection. e.g. In identification of underground insect pests, there are several items as follows: (1) classification of soil insect, (2) identification of grubs, (3) identification of wire worms, (4) identification of mole crickets, (5) identification of cutworms, (6) identification of Tenebrionidae, (7) identification of veevils. If you are given an soil insect, belonging to the group of grubs as you know, you can identify it on the level of species using item (2), on the other hand you can also classify it firstly into a certain group of pests using item (1), without knowing which group it belongs to and then continue to identify it on the level of species. In the procedure of identification, the system also has the function of searching backward, thus it can correct errors in time and draw a conclusion quickly.

24-034

STUDY ON ESTABLISHMENT RISK OF CODLING MOTH, *CYDIA POMONELLA* (L.), IN CHINA BY USING GEOGRAPHICAL INFORMATION SYSTEM

LIN Wei, ZHANG Cong-zhong

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The method on application of Geographical Information System (GIS) on Pest Risk Analysis (PRA) was developed. Data on selected variables that influence the geographic distribution of codling moth *Cydia pomonella* (L.) were assembled and entered into a computerized geographical information system to study relationship between climatic factors and geographical distribution of codling moth. The studies showed geographic distribution of this pest in latitude might be determined by temperature and temperature sum and geographic distribution in longitude had close relationship with rainfall from June to September. Depending on above variables, the preliminary forecasting results indicated the potential suitable establishment area located mainly in the west of China and the part of northeast of China. The potential unsuitable establishment area located mainly in the south of China and the part of east of China.

24-033

COMPUTERIZED EXPERT SYSTEM FOR IDENTIFICATION OF THE FAMILIES OF CHALCIDOIDEA (HYMENOPTERA)

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Computerized expert systems for taxon identification have many advantages over traditional identification devices, such as dichotomous keys. For the developer, one of the most important feature of such systems is that the addition or exclusion of characters and taxa can be done without the entire modification of the system structure. For the user, the most obvious advantage is the absence of the author-defined order of characters: in every step of the identification the user can select any of the available characters and skip the ones that cannot be observed or are uncertain. With the increasing availability and affordability of the computer technology, especially of notebook computers, it is very likely that expert systems will soon replace traditional methods of taxa identification.

The presented computerized identification system was developed using the Delta and Intkey for Windows software packages, and allows the identification of the families of the superfamily Chalcidoidea - a large and important group of parasitic Hymenoptera.

24-035

TAKEING CHIGGER (TROMBICULIDAE: ACARI) MORPHOLOGIC AND TAXONOMIC STUDIES INTO THE TWENTY-FIRST CENTURY.

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It has been proposed that studies on the ultrastructure of members of the family Trombiculidae of the sub-class Acari be carried out. The objective being to enhance our knowledge of morphological characteristics of general and specialized structures of this acarine group. With enhanced clarity of morphology the specialized structures could be more accurately evaluated and taxonomic relationships better assessed.

The proposed studies would encompass the comparison of phase contrast photomicrographs, illustrations and measurements with scanning electron microscope photomicrographs, establishment of morphologic character and character states, and utilization of classical chigger taxonomy and cladistic analysis to determine new taxa or synonymies.

24-036

EXPORT QUARANTINE STATUS OF *ATHERIGONA ORIENTALIS* (MUSCIDAE DIPTERA) ON FRESH HORTICULTURAL COMMODITIES FROM AUSTRALIA.

S. Schlack, N.W. Heather, E. Hassan, University of Queensland
Gatton College, Lawes Australia.

Market access of Australian tomatoes and cucurbits to New Zealand has been impeded by restrictions relating to the presence of eggs of *Atherigona orientalis* Schiner on fruit. Trials were conducted on the incidental effects of fruit fly treatments with the insecticidal dips with dimethoate and fenthion. Natural mortality was high supporting those literature reports which categorised the species as saprophage. Overall, the combination of incidental effects of fruit fly treatments with insecticides, together with normal mortality gave an assurance that egg numbers would be <99.5%. When viability of eggs present at pre-export inspection is corrected for this mortality, most produce grown is in accordance with good agricultural practice set out in the Bilateral Quarantine Agreement between the two countries and can meet the New Zealand permissible tolerance of <1 infected fruit in 200. The uncertainty over whether the pest is saprophagous or phytophagous is discussed in terms of the results recorded.

24-037

MORTALITY RESPONSE OF TWO COOK ISLAND FRUIT FLY (DIPTERA: TEPHRIIDAE) SPECIES TO HOT WATER IMMERSION

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Changes in the quarantine treatment technology used for disinfecting Cook Island fruit were necessitated by the lowering of the maximum allowable residue of ethylene dibromide detected in fumigated papaya destined for the New Zealand market. Postharvest fumigation was replaced by heat treatment which has itself been widely investigated as an alternative to chemical treatments particularly for tropical fruits. As a first step in developing this postharvest heat treatment it was necessary to identify the relative heat tolerance of the various life stages of the various pest species that may potentially be found on or in the fruit at the time of harvest. Once identified, this most tolerant species/life stage was included in all subsequent research to develop the treatment parameters that will be effective in killing species of quarantine concern without damaging the fruit.

In this study the mortality responses of two species of fruit fly *Bactrocera melanotus* (Coquillett) and *B. xanthodes* (Broun) to heat treatments were investigated. Temperatures in the range 43-48°C were used to test the response of eggs (<6 h, >24 h), first instar, second instar, and third instars (young, mid, old) to hot water immersion. The most heat tolerant life stage was >24 h eggs followed by first instars, third instars (of all ages), and finally <6 h eggs which were least tolerant. Species comparisons showed that *B. melanotus* eggs were more heat tolerant than *B. xanthodes* eggs while the reverse was true for larvae. Overall *B. melanotus* >24 h eggs were significantly more heat tolerant than all other life stages of both species tested.

24-038

THERMAL DEATH KINETICS IN EGGS AND THIRD INSTAR OF THREE SPECIES OF FRUIT FLIES (DIPTERA: TEPHRIIDAE)

DUARTE, A.L.¹; JANG, E.B.²; MALAVASI, A.¹

¹ Dept. Biologia, Inst. Biociências, USP, São Paulo, Brazil; ² ARS/USDA,
Hilo, Hawaii, USA

The kinetics of thermal death and death rate curves for eggs and third instar larvae of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann); the South American fruit fly, *Anastrepha fraterculus* (Wiedemann) and the West Indian fruit fly, *A. obliqua* (Macquart) were determined at temperatures between 43 and 48°C and for times of up to 160 min using a hot water-immersion technique. Both eggs and larvae of all three species showed mortality that was directly related to the severity of treatment and time of exposure. The curves of survivorship exhibited nonlogarithmic death that were characterized by an initial lag in mortality followed by an increasing death rate with time at a given temperature. Eggs were more resistant to heat than larvae at a given temperature. *A. fraterculus* eggs and larvae appeared more sensitive to heat than those of either of *C. capitata* and *A. obliqua*. This information may be useful in determining suitable commodities attacked by these insects.

Italian Entomology

25-001

LIST OF THE ENTOMOLOGICAL COLLECTIONS HELD IN ITALIAN PUBLIC INSTITUTIONS

R. Poggi¹, C. Conci²

1 Museo Civico di Storia Naturale "G. Doria", Genova, Italy - 2 Museo Civico di Storia Naturale, Milano, Italy

A list of the public entomological collections stored in Italy is presented and briefly commented.

This is the first attempt to gather in one paper the data on Italian collections (which now are scattered in the literature or quite unpublished), with the aim to provide a helpful guide for the location of old and recent entomological materials.

A series of information is given for all the collectors and for those travellers who enriched the Italian Institutions with significant series of specimens. The private collections of living people are not quoted, except for the parts which already became of public property.

25-002

ICONOGRAPHY OF ITALIAN ENTOMOLOGISTS, WITH ESSENTIAL BIOGRAPHICAL DATA

C. Conci¹, R. Poggi²

1 Museo Civico di Storia Naturale, Milano, Italy -

2 Museo Civico di Storia Naturale "G. Doria", Genova, Italy.

The work will be a homage to the persons who in Italy worked in Entomology. It is reported the iconography of about 400 no-living persons who published papers, mostly taxonomic or faunistic, on pure or applied Entomology. It also includes some persons who made only collections which are preserved in public Institutes, and also a few foreign Entomologists who made important collections in Italy.

For comments and complements to Iconography, the essential biographical data of the figured, and also of many not figured, persons are reported. Altogether, about 600 names are commented.

The bibliography is limited to the principal one subsequent to the work of Conci (1975).

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